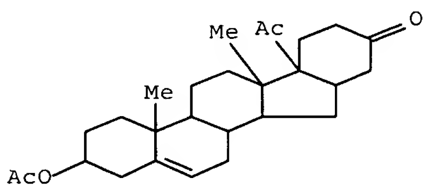
APPENDIX A

Attached are search results for documents disclosing Compounds II-VIII of Applicants' claimed invention and selected references.

Compound (II)

CAS Registry Record:

L1 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2006 ACS on STN
RN 83117-73-1 REGISTRY
ED Entered STN: 16 Nov 1984
CN 16,24-Cyclo-21-norchol-5-en-23-one, 17-acetyl-3-(acetyloxy)-,
(3β,16β,17α)- (9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN 9H-Indeno[2,1-a]phenanthrene, 16,24-cyclo-21-norchol-5-en-23-one deriv.
MF C27 H38 O4
LC STN Files: BEILSTEIN*, CA, CAPLUS, USPATFULL
(*File contains numerically searchable property data)

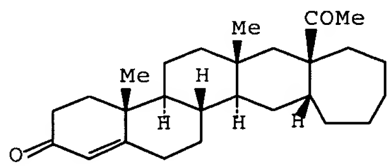


4 REFERENCES IN FILE CA (1907 TO DATE)
4 REFERENCES IN FILE CAPLUS (1907 TO DATE)

3 Lit Refs in Chem. Abs.; all in Russian:

<there are patent refs but newer than 2000>

L4 ANSWER 1 OF 3 HCA COPYRIGHT 2006 ACS on STN
AN 104:162095 HCA Full-text
TI Biological activity of transformed steroids. XXI. Synthesis, conformational analysis and biological activity of the D'7-pentarane, 16α,17α-cycloheptanoprogesterone
AU Kamernitskii, A. V.; Levina, I. S.; Kulikova, L. E.; Shamovskii, I. L.; Korkhov, V. V.; Nikitina, G. V.
CS Inst. Org. Khim., Moscow, USSR
SO Khimiko-Farmatsevticheskii Zhurnal **(1986)**, 20(1), 56-9
CODEN: KHFZAN; ISSN: 0023-1134
DT Journal
LA Russian
GI



I

AB 16α,17α-Cycloheptanoprogesterone (I) [101346-79-6] was synthesized; its conformation was described; and it was tested for progestogenic activity. I did not affect endometrium proliferation. This contrasted with the D'3-D'6-pentarane which did posses hormonal activity.

L4 ANSWER 2 OF 3 HCA COPYRIGHT 2006 ACS on STN

AN 98:179735 HCA Full-text

TI Transformed steroids. 131. Ring D' homologation in 16α,17α-cyclohexanopregnanes (D'6-pentaranes)

AU Kamernitskii, A. V.; Kulikova, L. E.; Levina, I. S.

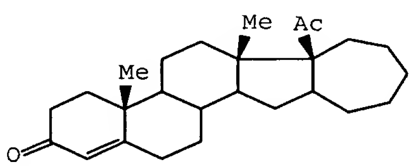
CS Inst. Org. Khim. im. Zelinskogo, Moscow, USSR

SO Izvestiya Akademii Nauk SSSR, Seriya Khimicheskaya **(1982)**, (11), 2552-7
CODEN: IASKA6; ISSN: 0002-3353

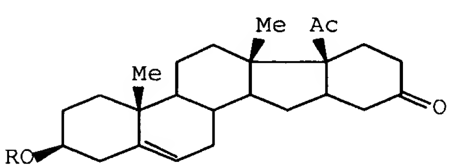
DT Journal

LA Russian

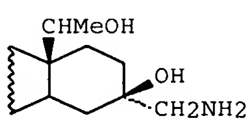
GI



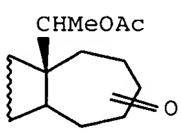
I



II



III



IV

AB The cycloheptanopregnane I was prepared from the cyclohexanopregnene II (R = Ac) in 9 steps. Thus, II underwent successive cyanation, acetylation, and LiAlH4 reduction to give the aminomethyl derivative III (R = H), which underwent Tiffeneau-Demjanov ring expansion and acetylation to give the cycloheptanopregnene IV. LiAlH4 reduction of the tosylhydrazone of IV and subsequent Jones oxidation gave I. Ring expansion of II via addition reactions of CH2N2 or CH2Br2 were not successful.

L4 ANSWER 3 OF 3 HCA COPYRIGHT 2006 ACS on STN

AN 97:163316 HCA Full-text

TI Transformed steroids. 125. Single-stage synthesis of oxo 16α,17α-cyclohexanopregnanes under atmospheric and high pressure

AU Levina, I. S.; Kulikova, L. E.; El'yanov, B. S.

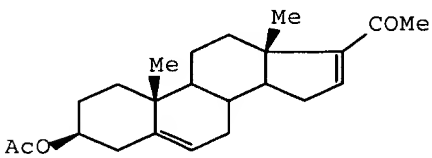
CS Inst. Org. Khim., Moscow, USSR

SO Izvestiya Akademii Nauk SSSR, Seriya Khimicheskaya (1982), (6), 1399-401
CODEN: IASKA6; ISSN: 0002-3353

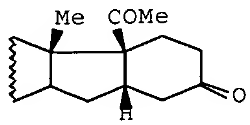
DT Journal

LA **Russian**

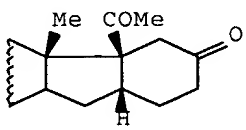
GI



I



II



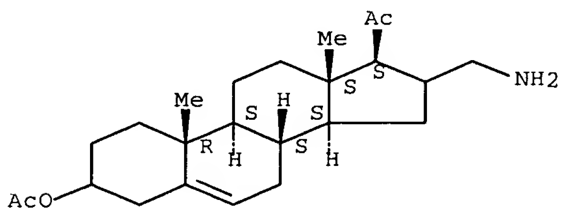
III

AB Cycloaddn. of the dehydropregnenolone acetate I with $H_2C:C(OSiMe_3)CH:CH_2$ in CH_2Cl_2 at 80° and 14 kbar gave a mixture of regioisomeric ketones II and III. Similar cyclization of I in the presence of $AlCl_3$ gave only II.

Compound (III)

CAS Registry Record:

RN 115605-73-7 REGISTRY
ED Entered STN: 06 Aug 1988
CN Pregn-5-en-20-one, 16-(aminomethyl)-3-hydroxy-, acetate (6CI) (CA INDEX NAME)
FS STEREOSEARCH
MF C24 H37 N O3
SR CAOLD
LC STN Files: BEILSTEIN*, CAOLD, CASREACT
(*File contains numerically searchable property data)
Absolute stereochemistry.



1 REFERENCES IN FILE CAOLD (PRIOR TO 1967)

1 Patent Ref in Chem. Abs:

L8 ANSWER 1 OF 1 CAOLD COPYRIGHT 2006 ACS on STN
AN CA51:16573b CAOLD
TI 16-(α-aminoalkyl)-4-pregnene-3,-20-diones
AU Dodson, Raymond M.
DT Patent
TI 16-(α-aminoalkyl)-4-pregnene-3,20-diones
PA Searle, G. D., & Co.
DT Patent

	PATENT NO.	KIND	DATE
PI	**US 2794815**		1957

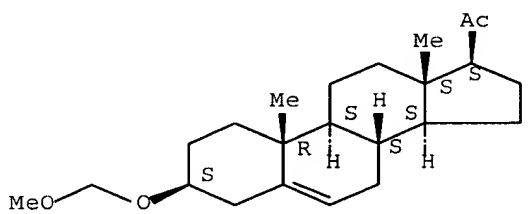
IT 102874-38-4 102945-38-0 103128-58-1 103511-98-4 113863-01-7
115605-73-7 117888-58-1 119111-97-6 119324-48-0 119599-03-0
119599-04-1 122871-87-8 123127-22-0 123267-90-3 124115-81-7 124263-31-6

Compound (IV)

CAS Registry Record:

RN 23328-05-4 REGISTRY
ED Entered STN: 16 Nov 1984
CN Pregn-5-en-20-one, 3-(methoxymethoxy)-, (3β)- (9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN Pregn-5-en-20-one, 3β-(methoxymethoxy)- (7CI, 8CI)
OTHER NAMES:
CN 3-O-Methoxymethyl-5-pregnen-3β-ol-20-one
CN NSC 64992
FS STEREOSEARCH
MF C23 H36 O3
LC STN Files: BEILSTEIN*, CA, CAOLD, CAPLUS, CASREACT, TOXCENTER, USPAT2, USPATFULL
(*File contains numerically searchable property data)

Absolute stereochemistry.



15 REFERENCES IN FILE CA (1907 TO DATE)
15 REFERENCES IN FILE CAPLUS (1907 TO DATE)
1 REFERENCES IN FILE CAOLD (PRIOR TO 1967)

Patent Refs in Chem. Abs.:

L10 ANSWER 1 OF 1 CAOLD COPYRIGHT 2006 ACS on STN
AN CA58:9193f CAOLD
TI (lower alkoxy)methyl ether derivs. of steroids
AU Fried, Josef
DT Patent
TI steroids, (lower alkoxy)methyl ether derivs. of
PA Olin Mathieson Chemical Corp.
DT Patent

	PATENT NO.	KIND	DATE
PI	**US 3062846**		1962

IT 2692-17-3 2692-42-4 2822-16-4 3109-81-7 4619-61-8
23328-05-4 105668-24-4

Oldest Lit Refs in Chem. Abs:

L14 ANSWER 10 OF 11 HCA COPYRIGHT 2006 ACS on STN
AN 96:5735 HCA Full-text
TI Alkyl-, aryl-, vinyl-, and heterosubstituted organozirconium compounds - selective nucleophiles of low basicity
AU Weidmann, Beat; Maycock, Christopher D.; Seebach, Dieter
CS Lab. Org. Chem., Swiss Fed. Inst. Technol., Zurich, CH-8092, Switz.
SO Helvetica Chimica Acta (1981), 64(5), 1552-7
CODEN: HCACAV; ISSN: 0018-019X

DT Journal
LA English
OS CASREACT 96:5735
AB Solns. of the title compds. are accessible from organolithium reagents and trialkoxyzirconium chloride. In contrast to their Ti analogs, vinylzirconium reagents are stable enough to be employed. Generally, organozirconium reagents are highly selective carbonylophiles of exceedingly low basicity for aldehydes and ketones.
CC 21-3 (General Organic Chemistry)
IT 853-23-6 15600-08-5 23328-05-4
RL: RCT (Reactant); RACT (Reactant or reagent)
(reaction of, with organozirconium compds.)

L14 ANSWER 11 OF 11 HCA
AN 73:35622 HCA

Full Text

TI Steroids and related natural products. XLVIII. Bufadienolides. 1. Introduction and base-catalyzed condensation of methyl ketones with glyoxylic acid
AU Pettit, George R.; Green, Brian; Dunn, George L.
CS Dep. of Chem., Arizona State Univ., Tempe, AZ, USA
SO Journal of Organic Chemistry (1970), 35(5), 1367-76
CODEN: JOCEAH; ISSN: 0022-3263
DT Journal
LA English
AB A comprehensive study of an aldol condensation between glyoxylic acid and various Me ketones was described. At high hydroxyl ion concentration, methyl β-naphthyl ketone gave bis(β-naphthacyl)acetic acid but by careful control of pH the condensation could be directed to yield trans-β-naphthoylacrylic acid and (or) a mixture of α-hydroxy-γ-oxobutyric acid and α-methoxy-γ- oxobutyric acid. The reaction was applied to Me cyclopentyl ketone, 2,5-dimethoxyacetophenone, 2,4-dimethylacetophenone, pinonic acid, and the steroidal ketones, 3β-hydroxy-20-oxo-5-pregnene and 3β-hydroxy-20-oxo-5α-pregnane.
CC 32 (Steroids)
IT 23328-04-3P 23328-05-4P 23328-06-5P 23328-07-6P
23328-08-7P 23328-09-8P 23328-10-1P 23328-11-2P 23328-12-3P
23328-13-4P 23328-15-6P 23328-16-7P 23328-17-8P 23328-18-9P
23330-45-2P 23349-18-0P 23349-19-1P 23349-20-4P 23349-21-5P
23349-22-6P 23349-23-7P 23349-24-8P 23349-25-9P 23349-26-0P
23349-27-1P 23359-85-5P 23389-68-6P
RL: SPN (Synthetic preparation); PREP (Preparation)
(preparation of)

(1 mm) in small round-bottomed flasks[2a,b] or inside the injection port of the gas chromatograph.[4] Relative yields of **3**, **4**, **5**, and **6** were determined by planimeter measurement of glpc peak areas and are reported in Scheme I. The yields of recovered **1**α, **1**β, **1**γ, and **1**δ relative to total methine were 23, 35, 98, and 96%, respectively, and their retention times were 19, 25, 25, and 26 min on the 10 ft $\times$ 0.25 in. Carbowax 20M column.[7] Under the same conditions, **3**, **4**, **5**, and **6** showed 18, 22, 20, and 19 min retention, respectively. Samples of **4** and **5** were purified by preparative gas chromatography.[7] Some spectral properties follow.

Methine **4**: ir (liquid film) 2950, 2850, 2775, 2750, 1460, 1380, 1260, 1040, 880, 845 cm^{-1}; $[\alpha]^{25}D$ +139° (*c* 0.2, $CHCl_3$); nmr[9] (neat) δ 4.8 (m, 2), 2.1 (s, 6), 2.0 (s, 2), 1.0 (d, 3, J = 6 Hz), and 0.7 (d, 3, J = 6 Hz). *Anal.* Calcd for $C_{13}H_{25}N$: C, 79.49; H, 12.79. Found: C, 79.17; H, 12.82.

(9) The nmr spectra were obtained on a Varian A-60 spectrometer with tetramethylsilane as internal standard. The infrared spectra were determined with a Beckman IR-5a spectrometer.

5:[2a,b] ir (liquid film) 2950, 2850, 2775, 2750, 1650 (6.05 μ),[2b,4a] 1460, 1380, 1270, 1050, 1030, 885 (11.30 μ);[2b,4a] nmr (CCl_4) δ 4.7 (m, 2), 2.1 (s, 6), 1.65 (m, 3), 0.85 (d, 3, J = 6 Hz).[10]

Peaks from the mass spectra of **3**, **4**, **5**, and **6** are reported in Table II.

Registry No.—**1**α, 2065-32-9; **1**β, 2232-27-1; **1**γ, 23912-39-2; **1**δ, 2883-89-8; **2'**α, 23912-41-6; **2'**γ, 23912-42-7; **2'**δ, 23912-43-8; **3**, 23912-44-9; **4**, 23912-45-0; **5**, 23912-46-1; **6**, 23912-47-2.

Acknowledgment.—We thank Dr. E. L. Eliel for his advice and many helpful suggestions, and Dr. O. C. Dermer, who also read the manuscript. The partial support of the National Research Foundation through GB-5607 and the American Petroleum Institute through API Research Project 58A is gratefully acknowledged.

(10) We acknowledge a prior nmr determination by H. R. Juneja.

Bufadienolides. 1. Introduction and Base-Catalyzed Condensation of Methyl Ketones with Glyoxylic Acid[1]

GEORGE R. PETTIT[2]

Department of Chemistry, Arizona State University, Tempe, Arizona 85281

BRIAN GREEN AND GEORGE L. DUNN

Department of Chemistry, University of Maine, Orono, Maine 04473

Received February 11, 1969

Introduction to a series of contributions pertaining to syntheses of isocardenolides, cardenolides, isobufadienolides, and bufadienolides is presented. A comprehensive study of an aldol condensation between glyoxylic acid and various methyl ketones is described. At high hydroxyl ion concentration, methyl β-naphthyl ketone gives bis(β-naphthacyl)acetic acid (**11a**), but by careful control of pH the condensation can be directed to yield the γ-ketoacrylic acid **16a** and/or a mixture of α-hydroxy-γ-oxobutyric acid (**15a**) and α-methoxy-γ-oxobutyric acid (**17a**). The reaction is applied to methyl cyclopentyl ketone, 2,5-dimethoxyacetophenone, 2,4-dimethylacetophenone, pinonic acid (**18**), and the steroidal ketones, 3β-hydroxy-20-oxo-5-pregnene (**7a**) and 3β-hydroxy-20-oxo-5α-pregnane (**24a**).

Ch'an su, the dried venom of a common Chinese toad, and extracts of the Mediterranean plant *Scilla maritima* (white squill) have received varied application in primitive medical practice for at least several millennia. The latter has been used from *ca.* 3500 B.C.[3] in the form of active glycoside extracts, principally for its diuretic and heart effects, but by the middle ages applications of the drug had gradually subsided. The heart effects were rediscovered in the early 18th century, but, with introduction of digitalis glycosides about 1785,[4] the plant was again gradually abandoned. The pioneering chemical investigations of Stoll[5] with the squill glycosides and Wieland[6] with extracts from the European toad *Bufo vulgaris* led, respectively, to structures for scillaren A,[7] bufotalin[8] (**1a**), and bufalin[9] (**1b**). The aglycones proved to be steroids bearing an α-pyrone ring at position 17 (*cf.* **1a**).[10,11]

Characteristic chemical and physiological[12] features of the plant and toad steroidal α-pyrones appear in bufalin (**1b**). In 1957, when the present study was initiated, neither bufalin nor any naturally occurring bufadienolide had yielded to total synthesis, and indeed no method was available for preparing even simpler 5-substituted 2-pyrones, such as **3**. Since then a preliminary account of the synthesis of a steroidal α-pyrone of the bufadienolide type has been reported,[13] and recently Sondheimer described a synthesis of

(1) Steroids and Related Natural Products. XLVIII. For the preceding contribution, see J. C. Knight and G. R. Pettit, *Phytochemistry*, **8**, 477 (1969). This investigation was supported by Public Health Service Research Grants CY-4074 (C3) to CA-04074-06 and CA-10115-01 to CA-10115-02 from the National Cancer Institute, and is based, in part, on the Ph.D. dissertation submitted in June 1962 by G. L. Dunn to the University of Maine.

(2) To whom correspondence should be addressed.

(3) F. M. Dean, "Naturally Occurring Oxygen Ring Compounds," Butterworth and Co. Ltd., 1963.

(4) A. Stoll, *Chem. Ind.* (London), 1558 (1959).

(5) A. Stoll, E. Suter, W. Kreis, B. B. Bussemaker, and A. Hofmann, *Helv. Chim. Acta*, **16**, 703 (1933).

(6) H. Wieland and F. J. Weil, *Chem. Ber.*, **46**, 3315 (1913).

(7) A. Stoll and J. Renz, *Helv. Chim. Acta*, **24**, 1380 (1941).

(8) For leading references, see K. Meyer, *ibid.*, **32**, 1993 (1949).

(9) Isolation and structural determination of bufalin was reported by K. Kuwada [*J. Chem. Soc: Jap.*, **60**, 335 (1939); *Chem. Abstr.*, **34**, 1031 (1940)] and was confirmed by K. Meyer [*Helv. Chim. Acta*, **32**, 1238 (1949)].

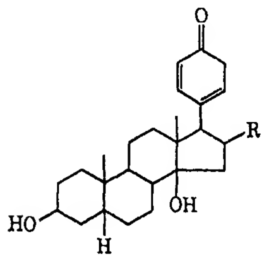
(10) In the case of hellebrigenin, the same aglycone has been found in both a plant extract and toad venom. For this and other interesting facets of bufadienolide chemistry, see ref 3 and other reviews cited therein.

(11) Subsequent extensive studies of Ch'an su, particularly by K. Meyer and colleagues, has led to location and identification of a number of related bufadienolides in this material, the most recent being 19-oxocinobufagin and 19-oxocinobufotalin: K. Meyer, *ibid.*, **52**, 1097 (1969).

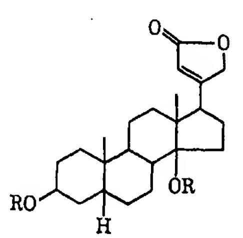
(12) The cardiac action of bufalin has been found almost equal to that of digitoxigenin (**2**) and in respect to local anesthetic potency on the rabbit cornea, *ca.* 90 times that of cocaine; see M. Okada, F. Sakai, and T. Suga, *Itsuu Kenkyusho Nempo*, **67**, 75 (1960); *Chem. Abstr.*, **55**, 16798 (1961). The bufadienolides generally display digitalis-like activity; *e.g.*, see K. K. Chen and A. Kovařikova, *J. Pharm. Sci.*, **56**, 1535 (1967); H. Murase, *Jap. J. Pharmacol.*, **15**, 72 (1965); *Chem. Abstr.*, **63**, 7517 (1965); W. Foerster, *Acta Biol. Med. Ger.*, **9**, 341 (1962); *Chem. Abstr.*, **58**, 11846 (1963).

(13) D. Bertin, L. Nedeloc, and J. Mathieu, *Compt. Rend.*, **253**, 1219 (1961).

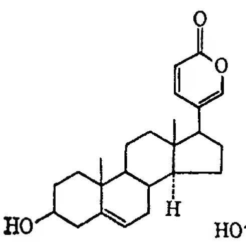
bufalin and resibufogenin.[14] With the objective of making bufadienolides more readily available for biological evaluation,[15] we decided to develop a practical synthesis of bufadienolides (*cf.* **3**) and complete a total synthesis of bufalin (**1b**). For reasons already apparent, the bufadienolide intermediates were also to be employed whenever appropriate for construction of cardenolide-type[4,16] lactones.



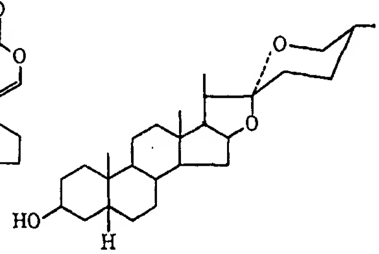
1a, R = $COOCH_3$
b, R = H



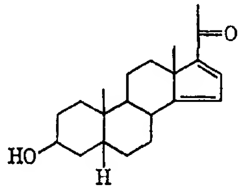
2



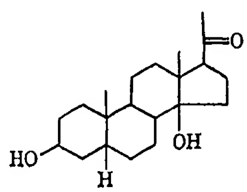
3



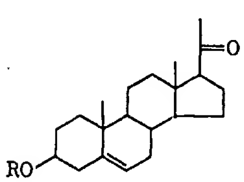
4

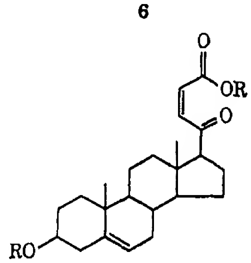


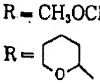
5



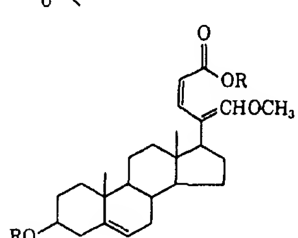
6





7a, R = H
b, R = $COCH_3$
c, R = $(C_6H_5)_3C$
d, R = CH_3OCH_2
e, R = 

8



9

As originally conceived, smilagenin (**4**) was to serve as relay for obtaining diene **5** (alternatively prepared by a total synthetic sequence) and then 20-oxopregnane **6**. Simultaneously, pregnenolone (**7a**) was to be used to develop a general synthesis of bufadienolides which could be applied to bufalin intermediate **6**. Degradation of smilagenin to diene **5** was readily accomplished,[17] and, with completion of a total synthetic route to the steroidal sapogenins by Sondheimer and colleagues, formal total synthesis in turn of diene **5** was at hand. Experiments in progress to convert diene **5** into 14β alcohol **6** were discontinued when the Meister[18] and Sondheimer[19] synthesis of digitoxigenin (**2**) presented the possibility of using the glycoside digitoxin as a starting point for total synthesis of bufalin. Meanwhile, transformation of pregnenolone to γ-ketoacrylic acid **8** was being explored as summarized below, with the object of entering ketone **8** in a Wittig reaction leading to vinyl ether **9**, as noted in part 4.[20] An acidification sequence was then expected to provide the corresponding α-pyrone. Before a satisfactory procedure was uncovered for obtaining **9**, the isobufadienolide and bufadienolide syntheses described in parts 6 and 7 were completed.[21] Reduction of ketone **8** to isocardanolide and isocardenolide systems did proceed as planned and culminated in the lactone syntheses described in parts 2 and 3.[22]

The pressing requirement for an efficient route to 20-oxo-21-nor-22-cholenic acids suggested the exploration of an aldol condensation between methyl ketones and glyoxylic acid, despite the fact that no practical one-step conversion of this type had been reported. Shortly afterward, Newman[23] described the base-catalyzed condensation of a glyoxylate with the cyclic ketone α-tetralone to give an analogous product.[24] Later, Noltes and Kögl[25] found that heating the diethyl

(14) F. Sondheimer, W. McCrae, and W. J. Salmond, *J. Amer. Chem. Soc.*, **91**, 1228 (1969). A review of 2-pyrone syntheses has been prepared: N. P. Shusherina, N. D. Dmitrieva, E. A. Luk'yanets, and R. Y. Levina, *Russ. Chem. Rev.*, **36**, 175 (1967).

(15) *Our interest in 1957 was strongly motivated by reports that certain* α,β-unsaturated lactones inhibit cell growth: L. J. Haynes, *Quart. Rev.* (London), **2**, 46 (1948). Since then, antitumor properties have been attributed to cardenolides [S. M. Kupchan, M. Mokotoff, R. S. Sandhu, and L. E. Hokin, *J. Med. Chem.*, **10**, 1025 (1967)], bufadienolides [S. M. Kupchan, R. J. Hemingway, and J. C. Hemingway, *Tetrahedron Lett.*, 149 (1968)], and other lactones [S. M. Kupchan, R. W. Doskotch, P. Bollinger, A. T. McPhail, G. A. Sim, and J. A. Saenz Renauld, *J. Amer. Chem. Soc.*, **87**, 5805 (1965); J. E. Pike, J. E. Grady, J. S. Evans, and C. G. Smith, *J. Med. Chem.*, **7**, 348 (1964)].

(16) T. Reichstein, *Naturwissenschaften*, **54**, 53 (1967).

(17) G. R. Pettit and D. M. Piatak, *Can. J. Chem.*, **44**, 844 (1966). Results of an analogous study have recently been reported: G. Bach, J. Capitaine, and C. R. Engel, *ibid.*, **46**, 733 (1968); R. Bouchard and R. Engel, *ibid.*, **46**, 2201 (1968).

(18) P. D. Meister and H. C. Murray, U. S. Patent 2,968,596 (1961); *Chem. Abstr.*, **55**, 11,466 (1961).

(19) N. Danieli, Y. Mazur, and F. Sondheimer, *Tetrahedron*, **22**, 3189 (1966).

(20) G. R. Pettit, B. Green, G. L. Dunn, and P. Sunder-Plassmann, *J. Org. Chem.*, **35**, 1385 (1970).

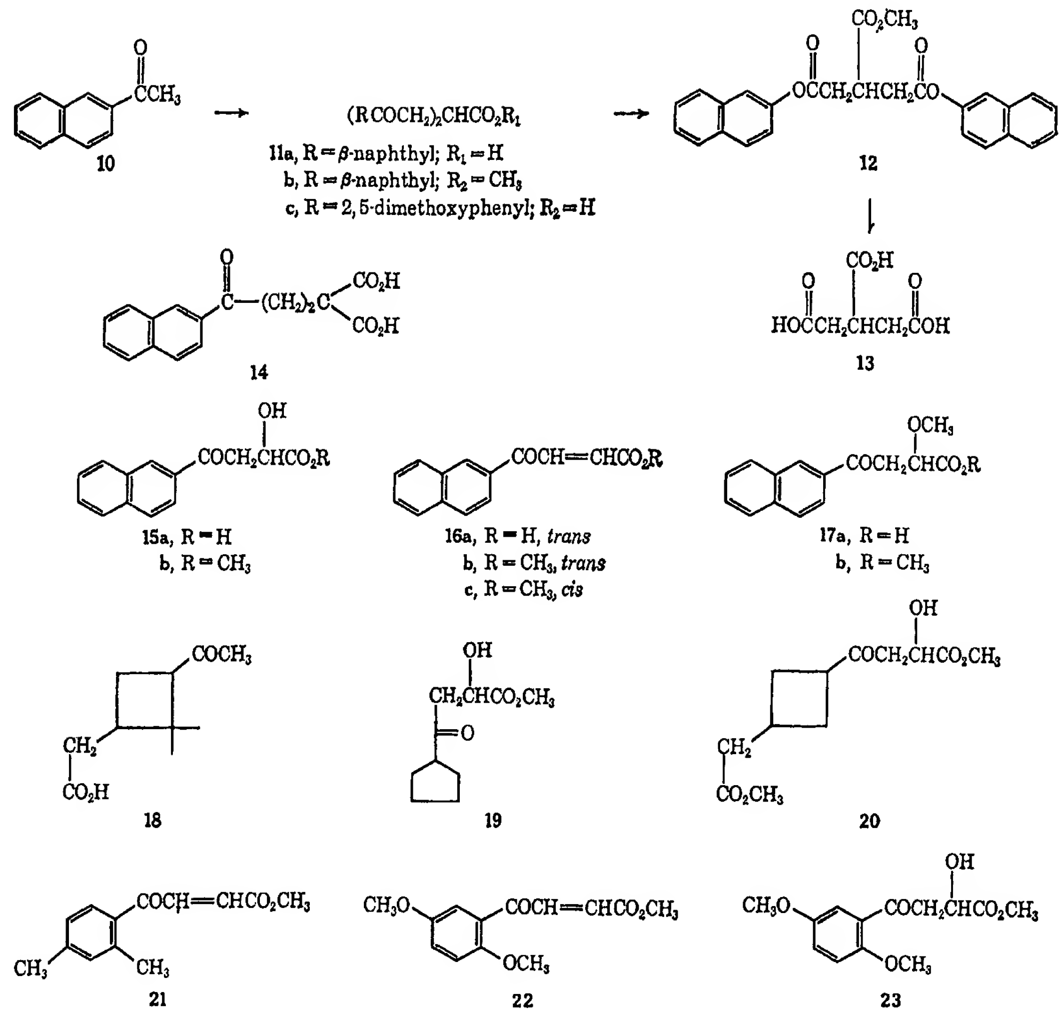
(21) (a) G. R. Pettit, J. C. Knight, and C. L. Herald, *ibid.*, **35**, 1393 (1970); (b) G. R. Pettit, D. Fessler, K. Paull, P. Hofer, and J. C. Knight, *ibid.*, **35**, 1398 (1970).

(22) (a) G. R. Pettit, B. Green, and G. L. Dunn, *ibid.*, **35**, 1377 (1970); (b) G. R. Pettit, B. Green, A. K. Das Gupta, P. A. Whitehouse, and J. P. Yardley, *ibid.*, **35**, 1381 (1970).

(23) M. S. Newman, W. C. Sagar, and C. C. Cochrane, *ibid.*, **23**, 1832 (1958).

(24) A study of the condensation of aqueous glyoxylic acid with 17-oxoandrostanes has been made: P. Kurath and W. Cole, *ibid.*, **26**, 1939 (1961). We wish to thank Dr. Kurath for allowing us to review this manuscript prior to publication.

(25) A. W. Noltes and F. Kögl, *Rec. Trav. Chim. Pays-Bas*, **80**, 1334 (1961); see also P. Kurath and W. Cole, *J. Org. Chem.*, **26**, 4592 (1961).



acetal of ethyl glyoxylate with a variety of ketones yielded α-hydroxy-γ-oxobutyric acid esters which could be dehydrated to the corresponding acrylic acids. Early attempts to condense glyoxylic acid with methyl ketones of the acetophenone type had yielded, instead of acrylic acid derivatives, bis(phenacyl)acetic acids,[26] presumably by Michael condensation of the initially formed acrylic acid with a second molecule of methyl ketone.

To determine whether a normal aldol condensation could occur, a number of reactions utilizing methyl β-naphthyl ketone[27] and glyoxylic acid or a glyoxylate were evaluated. Prior to appearance of the Newman procedure,[23] glyoxylic acid and aqueous solutions of the acid were prepared by treating barium glyoxylate with sulfuric acid. Concurrently, the more readily characterizable butyl glyoxylate (from lead tetraacetate cleavage of di-*n*-butyl tartrate) was also employed. Among a variety of acid-[28] and base-catalyzed[29] aldol conditions studied, only those reactions employing butyl glyoxylate and methyl β-naphthyl ketone, in ethyl alcohol containing 10% aqueous sodium hydroxide, gave reasonable amounts of acidic condensation products. The colorless carboxylic acid $C_{26}H_{20}O_4$ obtained was the product (**11a**) arising from Michael addition[26] of ketone **10** to the initially formed γ-keto-acrylic acid, and conclusive evidence for the bis(β-naphthacyl)acetic acid structure was obtained as follows. Acid **11a** was methylated with diazomethane and ester **11b** was treated with peroxytrifluoroacetic acid.[30] The resulting triester **12** was saponified and, following acidification, both β-naphthol and tricarballylic acid (**13**) were isolated. Assignment **11a** was further supported by an unequivocal synthesis in which diethyl malonate was condensed with ω-bromo-2-acetonaphthone and the product was saponified to provide the disubstituted malonic acid **14**, which on partial decarboxylation gave acetic acid derivative **11a**.[31]

Repeating the aldol route to acid **11a** in aqueous tetrahydrofuran–methanol at pH 14 using glyoxylic acid prepared[23] *in situ* from tartaric acid afforded bis(β-

(26) M. J. Bougault, *Compt. Rend.*, **148**, 1270 (1909).

(27) Structure of the acrylic acid **16** which would be obtained from this ketone was firmly established: G. Baddeley, G. Holt, S. M. Maker, and M. G. Ivinson, *J. Chem. Soc.*, 3605 (1952); M. Goldman and E. I. Becker, *Nature*, **170**, 35 (1952); *Chem. Abstr.*, **48**, 116 (1954).

(28) Z. Csuros, J. Petro, and P. Konig, *Acta Chim. Acad. Sci. Hung.*, **17**, 419 (1958); *Chem. Abstr.*, **53**, 17,053 (1959).

(29) For a comprehensive review of the aldol condensation, see A. T. Nielsen and W. J. Houlihan, *Org. React.*, **16**, 1 (1968).

(30) W. D. Emmons and G. D. Lucas, *J. Amer. Chem. Soc.*, **77**, 2287 (1955).

(31) An analogous sequence has been used to prepare bis(phenacyl)-acetic acid: W. Kues and C. Paal, *Chem. Ber.*, **19**, 3144 (1886).

naphthacyl)acetic acid in 65% yield. Under these strongly alkaline conditions, 2,5-dimethoxyacetophenone was easily transformed into acetic acid derivative **11c**. Application of conditions similar to those of Newman,[23] *i.e.*, lower pH, gave α-hydroxy-β-(2-naphthoyl)propionic acid (**15a**, 23%) accompanied by a lesser quantity of β-(2-naphthoyl)acrylic acid (**16a**). Heating the α-hydroxy acid in acetic anhydride with potassium hydrogen sulfate[25] gave acrylic acid **16a** in 33% yield.

Meanwhile, attempts were being made to condense aqueous glyoxylic acid obtained by the Newman method[23] with methyl ketones of the 20-oxopregnane type at various pH levels as noted below, and values (pH meter) of 13.25–13.65 were found most useful in effecting only the aldol condensation and avoiding further reaction to disubstituted acetic acids. Best conversion into acidic products was obtained at pH 13.65 (meter) in tetrahydrofuran–methanol containing 8% aqueous potassium hydroxide for 3 days at room temperature. By this means ketone **10** was transformed in up to 90% conversion into a mixture of three acids, which were esterified and separated by chromatography to give methyl esters **16b** (17%), **17b** (57%), and **15b** (10%). The unexpected ester **17b** exhibited strong infrared absorption at 1130 cm^{-1} characteristic of the carbon–oxygen bond in aliphatic ethers[32] and was deduced to be the product of addition of methanol to acrylic acid **16a**. Elemental analyses gave further support, and alternate preparation by methylation of α-hydroxy ester **15b** using diazomethane–boron trifluoride provided the necessary confirmation.[33]

Extension of the aldol condensation with glyoxylic acid to methyl cyclopentyl ketone and pinonic acid (**18**) gave comparable results, but only the α-hydroxy esters were characterized. Following methylation (diazomethane), the acid(s) from methyl cyclopentyl ketone and pinonic acid yielded α-hydroxy esters **19** (52%) and **20** (72%), respectively, as oils.

The principle objective, efficient conversion of ketone **10** into acrylic acid **16a**, was eventually achieved by allowing the aldol condensation to proceed for 12 hr at reflux temperature. The acidic products obtained in this way from β-naphthyl ketone, 2,4-dimethylacetophenone, and 2,5-dimethoxyacetophenone were methylated to furnish acrylates **16b**, **21**, and **22** in yields of 56–59%. The relative proportions of both aldol intermediates and methanol addition products were considerably smaller, as shown by careful investigation of the products from 2,5-dimethoxyacetophenone, which led to isolation of methyl α-hydroxy-β-(2,5-dimethoxybenzoyl)propionate (**23**) in 9% yield. Thin layer chromatography indicated the presence of a very small amount of the α-methoxy ester.

Shortly after the glyoxylic acid reaction with methyl ketones had reached a practical state of development, Bestmann reported[34] a valuable synthesis of γ-keto acrylic acids based on the condensation of an α-bromo ketone with carbomethoxymethylenetriphenylphosphorane. Application of this reaction to ω-bromo-2-acetonaphthone gave *trans*-methyl acrylate **16b** in 38% conversion. Irradiation of a solution of this yellow[35] product gave the colorless *cis* isomer **16c**. Side-chain olefin protons of the yellow isomer exhibited a coupling constant of 15 cps whereas the colorless isomer gave in the same region J = 11 cps, consistent with the assigned configurations.[36] While this general study of aldol-type reactions involving methyl ketones and glyoxylic acid was being undertaken, the model experiments now summarized were also being conducted.

Aldol condensation between benzaldehyde and 20-oxopregnenes using, *e.g.*, sodium methoxide in methanol, follows the predicted course and presents no problem.[37] However, a considerable number of experiments directed at condensing butyl glyoxylate or glyoxylic acid with 20-oxopregnanes **24a** or **7a** were quite unrewarding, leading in most cases to almost complete recovery of starting material. When the more general study of methyl ketones began to focus on aqueous glyoxylic acid prepared from tartaric acid,[23,24] it was decided to apply this method to suitable 5α- and Δ^5-20-oxopregnanes, which were chosen as models for the less readily available 3β-hydroxy-20-oxo-5β-pregnanes. It was originally deemed advisable to protect the 3β-hydroxyl group with a base-stable, acid-labile group, and those evaluated will now be discussed.

Triphenylmethyl chloride in pyridine solution generally favors reaction with a primary alcohol, but substitution of triphenylmethyl bromide can provide, in the case of secondary alcohols, greater than 80% yields of trityl ethers.[38] Modification (24-hr reaction period) of the Stegerhock procedure[38] with secondary alcohol **24a** and triphenylmethyl bromide gave reasonable conversion (53%) into ether[39] **24c**. Similarly, trityl ethers **7c** and **25b** were obtained in comparable yields, but low solubility of the trityl ethers in water–methanol–tetrahydrofuran mixtures caused rejection of this protecting group. In the expectation of a solubility increase in such protic solvent mixtures for a methoxymethyl ether,[40] a specimen of **7d** was obtained (18% yield) by treating pregnenolone (**7a**) with chloromethyl ether in the presence of silver oxide.[41] The

(32) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd ed, John Wiley & Sons, Inc., New York, N. Y., 1958.

(33) Alcohol **15a** may represent that portion of the aldol intermediate which did not undergo dehydration, although addition of water to the α,β-unsaturated carbonyl system after dehydration would still be expected to give the α- rather than the β-hydroxy acid. Hydrolysis of methyl β-(*p*-bromobenzoyl)crotonate in hot aqueous methanolic potassium hydroxide solution has been shown to yield α-hydroxy-β-(*p*-bromobenzoyl)butyric acid: W. Koenigs and E. Wagstaffe, *Chem. Ber.*, **26**, 554 (1893). Later experiments of a similar nature with methanol gave comparable results. See, *e.g.*, E. R. H. Jones, T. Y. Shen, and M. C. Whiting, *J. Chem. Soc.*, 236 (1950).

(34) H. J. Bestmann and H. Schulz, *Angew. Chem.*, **73**, 620 (1961). Experimental details of the reaction were kindly provided by Professor Bestmann prior to publication. Later a full report and review of this useful reaction were made available: H. J. Bestmann, *Angew. Chem. Int. Ed. Engl.*, **4**, 583 (1965).

(35) Previous study of *cis–trans* isomers in β-aroylacrylic acids suggested that the yellow geometrical isomer of acid **7a** could be assigned the *trans* configuration. For a pertinent summary, refer to ref 27 (M. Goldman, *et al.*).

(36) A preliminary account of these stereochemical assignments has been summarized: G. R. Pettit, B. Green, A. K. Das Gupta, and G. L. Dunn, *Experientia*, **20**, 248 (1964).

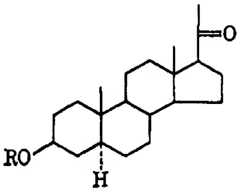
(37) I. Dory and G. Lanyi, *Acta Chim. Acad. Sci. Hung.*, **30**, 71 (1962); *Chem. Abstr.*, **58**, 564 (1963).

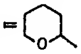
(38) L. J. Stegerhock and P. E. Verkade, *Rec. Trav. Chim. Pays-Bas*, **75**, 143 (1956).

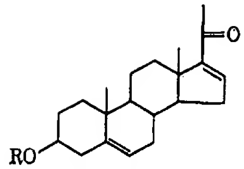
(39) For a related example, see, H. M. E. Cardwell, J. W. Cornforth, S. R. Duff, H. Holtermann, and R. Robinson, *J. Chem. Soc.*, 361 (1953).

(40) R. Stern, J. English, Jr., and H. G. Cassidy, *J. Amer. Chem. Soc.*, **79**, 5797 (1957). A steroid 11-methoxymethyl ether has been prepared using formaldehyde, methanol, and hydrochloric acid: R. E. Beyler, F. Hoffman, R. M. Moriarty, and L. H. Sarett, *J. Org. Chem.*, **26**, 2421 (1961).

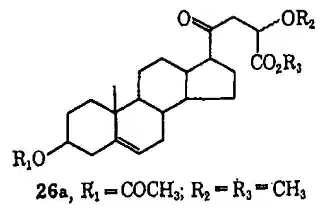
(41) G. R. Pettit and T. R. Kasturi, *ibid.*, **26**, 4553 (1961).

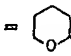


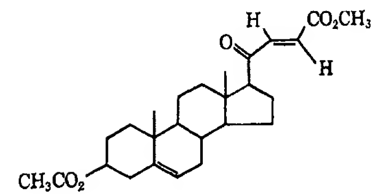
24a, R = H
b, R = $COCH_3$
c, R = $(C_6H_5)_3C$
d, R = 



25a, R = H
b, R = $(C_6H_5)_3C$



26a, $R_1 = COCH_3$; $R_2 = R_3 = CH_3$
b, $R_1 = R_2 = H$; $R_3 = CH_3$
c, $R_1 = R_2 = COCH_3$; $R_3 = CH_3$
d, $R_1 = R_2 =$  ; $R_3 = CH_3$



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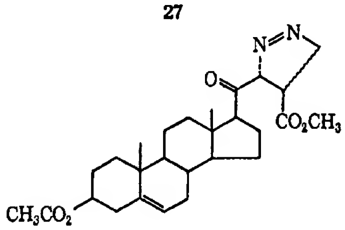

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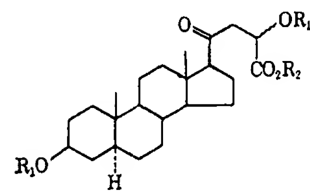


29a, $R_1 = R_2 = H$
b, $R_1 = H$; $R_2 = CH_3$
c, $R_1 = COCH_3$; $R_2 = CH_3$

solubility of ether **7d** and the more efficiently prepared tetrahydropyranyl ethers **24d** and **7e** proved quite favorable, but protecting-group studies were discontinued when use of blocked alcohols in the aldol reactions proved unnecessary.

Meanwhile, effects of potassium hydroxide concentration on the total yield of acidic material from reaction between pregnenolone and aqueous glyoxylic acid at various pH levels were being evaluated. The yield of acidic products increased substantially from 25% at a pH reading of 13.25 to 45% at 13.48 to 81% at 13.65. The procedure involved adding 8% aqueous potassium hydroxide to a solution composed of methanol, tetrahydrofuran, pregnenolone, glyoxylic acid, and water until the required pH scale reading was reached. The acidic product from a reaction at pH 13.48 was methylated with diazomethane and the crude mixture of methyl esters was separated by chromatography. A fraction eluted by benzene–chloroform was acetylated and rechromatographed to give methyl 3β-acetoxy-23-methoxy-20-oxo-21-nor-5-cholenate (**26a**, 20%), presumably arising by a Michael-type addition of methanol to the aldol condensation product. In addition to elemental composition and spectral data, the structure of ether **26a** was assigned using evidence already reviewed for the analogous product obtained from methyl β-naphthyl ketone.

A fraction eluted with chloroform gave methyl 3β-23-dihydroxy-20-oxo-21-nor-5-cholenate (**26b**, 30%), which was converted into both diacetate **26c** and bistetrahydropyranyl ether **26d**. Formation of these derivatives, combined with information already compiled for the aldol intermediate from methyl β-naphthyl ketone, provided structural evidence for diol **26b**.

The aldol reaction with pregnenolone and glyoxylic acid was repeated at a pH meter reading of 13.65 (optimal base concentration) and studied with respect to time and temperature. After 28 hr at room temperature, the acidic product was isolated, methylated, acetylated, and purified by column chromatography to give methyl 3β-acetoxy-20-oxo-21-nor-5-*trans*-22-choladienate (**27**),[42] methyl ether **26a**, diacetate **26c**, and an oily substance tentatively assigned pyrazoline structure **28** (formed by a 1,3-dipolar cycloaddition of diazomethane to the α,β-unsaturated ketone system).[43] Yields of the first three compounds amounted to, respectively, 5, 11, and 15%.

Extending the condensation to 72 hr increased the total yield of acidic products from 52 to 72%, and, following methylation, acetylation, and purification, olefin **27**, methyl ether **26a**, and diacetate **26c** were obtained in 8, 14, and 19% conversion, respectively. Adjusted for recovered pregnenolone, the respective yields were 15, 18, and 25% accompanied by 6% of the crude pyrazoline. It is apparent that increasing the reaction time increased the total yield of acidic products, but did not markedly affect the proportion of each constituent. Application of this procedure to 3β-acetoxy-20-oxo-5α-pregnane (**24b**) led to 3β,23-dihydroxy-20-oxo-21-nor-5α-cholanic acid (**29a**) as major product, whose structure was confirmed by preparation of the methyl ester (**29b**) and diacetate (**29c**) derivatives; no attempt was made to characterize the minor reaction constituents, which were presumably analogous to those obtained from ketone **7a**. Some indication of the necessity of controlling the base concentration was obtained by treating alcohol **29b** with 5% potassium hydroxide in methanol for a 2-hr period at reflux, at which time *ca.* 25% of the alcohol had undergone reverse aldol condensation.

(42) Evidence for the structure and stereochemistry of olefin **5** has been summarized in a preliminary communication: G. R. Pettit, B. Green, A. K. Das Gupta, and G. L. Dunn, *Experientia*, **20**, 248 (1964). A more complete summary is presented in part III.[22b]

(43) Similar reaction products have been investigated: See E. R. H. Jones, *et al.*, ref 33.

The parallel survey of reactions between glyoxylic acid and methyl β-naphthyl ketone by this time had shown that heating the aldol reaction mixture at reflux would increase acrylic acid formation. When pregnenolone was analogously condensed with glyoxylic acid, followed by methylation and acetylation, the yield of methyl ether **26a** and diacetate **26c** fell to, respectively, 1 and 8%. Substituting dimethoxypropane[44] for diazomethane in the methylation step allowed isolation of olefin **27** in *ca.* 20% yield.

Upon reaching this more promising stage for synthesis of γ-keto acrylic acid **27** by an aldol sequence, we were able to meet requirements for this compound by application of the then newly discovered Bestmann reaction.[34] However, the carefully defined experimental conditions reported herein for condensing glyoxylic acid with methyl ketones should prove of value where the α-halo ketone required for the Bestmann procedure cannot easily be obtained or where an aldol intermediate such as diol **29a** is required.

Experimental Section

Ligroin refers to a fraction boiling at 65-70°. Benzene and dihydropyran were redistilled from sodium; pyridine was redistilled from potassium hydroxide. Solvent extracts of aqueous solutions were dried over anhydrous sodium sulfate or magnesium sulfate. Acetylation reactions were conducted using 1:1 acetic anhydride-pyridine at room temperature for 14-20-hr periods. The basic (Alcoa grade F-20), neutral (E. Merck, Darmstadt), and acid-washed (Merck, Rahway, N. J.) aluminas were used as supplied. Melting points reported for analytical specimens were observed using a Kofler melting point apparatus. All other melting points were determined in open capillaries in a silicone oil bath and are uncorrected. The thin layer chromatograms were prepared on silica gel G (developed with concentrated sulfuric acid) or silica gel HF_{254} (both from E. Merck). All analytical specimens were checked for purity by thin layer chromatography. A Beckman zeromatic pH meter equipped with a Beckmann E-2 glass electrode and a calomel reference electrode was used for pH measurements.

Ultraviolet, infrared (potassium bromide pellets unless noted differently), and nuclear magnetic resonance (Varian A-60 spectrometer) spectra were recorded by Dr. R. A. Hill, University of Maine. The nuclear magnetic resonance data are expressed in parts per million (δ) downfield from tetramethylsilane. The microanalyses were provided by Dr. A. Bernhardt, Max Planck Institute, Mülheim, Germany, and by the laboratory of Dr. C. Janssen, Beerse, Belgium. Optical rotation measurements (chloroform solution) were provided by Dr. Weiler and Dr. Strauss, Oxford, England.

Glyoxylic Acid.—Unless otherwise stated, glyoxylic acid was prepared in the following manner and used immediately without further purification or isolation. To a cooled solution of tartaric acid (5.7 g, 0.038 mol) in water (9 ml) at 0° was added a solution of paraperiodic acid (8.6 g, 0.038 mol) in water (18 ml). Before this glyoxylic acid solution was used, the cleavage reaction was allowed to proceed for 12 min.

Bis(β-naphthacyl)acetic acid (11a). Method A.—To a solution of methyl β-naphtyl ketone (**10**, 2 g, 0.012 mol) in ethyl alcohol (10 ml, 95%) was added *n*-butyl glyoxylate[45] (2 g, 0.015 mol) followed by aqueous sodium hydroxide (10%, 16 ml, 0.04 mol). The mixture was heated for 6 hr at 65-70°, cooled, diluted to 100 ml with water, and extracted with diethyl ether. Acidification of the aqueous layer gave a pale yellow solid, mp 195-198°. Recrystallization from dioxane-water gave colorless plates (1.5 g, 63%), mp 198-199°. Three crystallizations from dioxane-water afforded the analytical specimen: mp 199-200°; ν_{max} 3400-2600 (carboxylic acid), 1700 (carboxyl group), and 1685 cm^{-1} (ketones).

Anal. Calcd for $C_{26}H_{20}O_4$ (mol wt, 396): C, 78.79; H, 5.10. Found: C, 78.23; H, 5.29; neut equiv, 374, 385.

Method B.—A rapidly stirred solution of methyl β-naphthyl ketone (**10**, 3.4 g, 0.02 mol) in tetrahydrofuran (35 ml)-methanol (50 ml) was treated successively with aqueous potassium hydroxide (12.6 g in 54 ml of water) and a solution of glyoxylic acid (0.012 mol) prepared by the general procedure described above. After addition of water (25 ml), a hydrogen ion measurement showed pH >14.0.

After 16 hr (with stirring) at room temperature, the solution phase was filtered to remove inorganic salts and evaporated *in vacuo* to the point of turbidity. Dilution with water (200 ml) and extraction with diethyl ether gave upon evaporation methyl β-naphthyl ketone (0.1 g). The aqueous suspension was acidified with concentrated hydrochloric acid and extracted with chloroform. Evaporation of the water-washed and dried extract gave the disubstituted acetic acid **11a** as a brown solid,[46] which crystallized from dioxane-water as leaflets (2.55 g, 65%), mp 192-193°.

Methyl Bis(β-naphthacyl)acetic Acid (11b).—To a solution of bis(β-naphthacyl)acetic acid (**11a**, 2.70 g) in dioxane (50 ml) was added excess ethereal diazomethane. The mixture was allowed to stand at room temperature for 6 hr. Diazomethane and ether were removed by warming and the remaining solution was diluted with water. The oil which separated crystallized upon trituration to yield a colorless solid (2.70 g), mp 95-97°. One recrystallization from dioxane-water raised the melting point to 116.5-117.5°. Recrystallization from the same solvent mixture gave an analytical specimen, mp 117.3-118°.

Anal. Calcd for $C_{27}H_{22}O_4$: C, 79.00; H, 5.40. Found: C, 79.05; H, 5.09.

Baeyer-Villiger Oxidation of Methyl Bis(β-naphthacyl)acetate (11b).—A solution of trifluoroperacetic acid prepared from trifluoracetic anhydride (1.7 ml, 0.012 mol) and hydrogen peroxide (90%, 0.27 ml, 0.010 mol) in methylene chloride (3 ml) was added with stirring during 15 min to a solution of methyl bis(β-naphthacyl)acetate (**11b**, 1 g, 0.0024 mol) in methylene chloride (15 ml) containing a suspension of dry disodium hydrogen phosphate (3.54 g, 0.005 mol). The yellow mixture was stirred at room temperature for 3 hr and at reflux for 7 hr. Filtration of the warm solution followed by evaporation furnished a yellow residue, which was dissolved in ethyl alcohol (95%, 5 ml) and heated at reflux for 3 hr with aqueous potassium hydroxide (20%, 4.5 ml, 0.015 mol). After evaporative removal of ethyl alcohol *in vacuo*, the aqueous solution was acidified with concentrated hydrochloric acid and the precipitated brown solid was collected (the filtrate was retained; see below) and washed with diethyl ether (two 10-ml portions). The ethereal extract was concentrated to a brown solid, mp 112-116°. One crystallization from water-methanol gave β-naphthol[46] as tan crystals (0.28 g, 40%), mp 119-121°.

The aqueous filtrate was evaporated to dryness and the residue was extracted with boiling chloroform (two 5-ml portions). Evaporation of the filtered chloroform solution furnished a tan solid (**13**, 0.10 g, 23%), mp 155-158°, which proved to be tricarballylic acid, mp 160-162°.

Alternate Synthesis of Bis(β-naphthacyl)acetic Acid (11a).—To a cooled (0°) solution of methyl β-naphthyl ketone (17 g, 0.10 mol) in dry diethyl ether (100 ml) was added, over 30 min, bromine (16 g, 0.10 mol). The brown ethereal solution was washed with water (four 75-ml portions), dried (sodium sulfate), and evaporated to a crystalline solid. Recrystallization from ethyl alcohol (95%) afforded plates (**14**, 14 g, 56%), mp 82-83°, of ω-bromo-2-acetonaphthone (lit.[47] mp 82.5-83.5°).

Absolute ethyl alcohol (38 ml) was added gradually to finely cut sodium (1.3 g, 0.057 mol). When hydrogen evolution was complete, diethyl malonate (9.1 g, 0.056 mol) was added to the vigorously stirred solution followed, during 20 min, by ω-bromo-β-acetonaphthone (14 g, 0.0562 mol) in hot absolute ethyl alcohol (100 ml). After a 2-hr period at reflux (with vigorous stirring), the ethyl alcohol was removed *in vacuo* and the oily brown residue was shaken with aqueous potassium carbonate (10%, 100 ml), followed by methyl alcohol (50 ml). The remaining residue was dissolved in hot benzene, filtered, and evaporated to a yellow oil which was heated at reflux for 2 hr with aqueous potassium hydroxide (20%, 50 ml). The mixture was cooled in ice and acidified with concentrated hydrochloric acid, and the acidic

(44) N. B. Lorette and J. H. Brown, Jr., *J. Org. Chem.*, **24**, 261 (1959).

(45) Prepared by cleavage of di-*n*-butyl tartrate with lead tetraacetate according to the method of Vogel: A. I. Vogel, "Practical Organic Chemistry," 3rd ed, Longmans, Green and Co., London, 1956, p 951.

(46) The structure was confirmed by mixture melting point determination and infrared spectral comparison with an authentic sample.

(47) T. Immediata and A. R. Day, *J. Org. Chem.*, **5**, 512 (1940).

product was collected by filtration. The air-dried **bis(β-naphthacyl)malonic acid** (14, 0.70 g) melted at 137–140° with decomposition.

The crude dicarboxylic acid (0.70 g) was heated at 150° until evolution of gas ceased (15 min), and the dark residue was dissolved in benzene, filtered, and treated with ligroin (1 ml). The brown solid (0.5 g, 79%) which precipitated melted at 193–195° and was identical with the acid 11a, obtained by condensing methyl β-naphthyl ketone with butyl glyoxylate.

Bis(2,5-dimethoxyphenacyl)acetic Acid (11c).—Using the procedure outlined above (method B), 2,5-dimethoxyacetophenone (3.6 g, 0.02 mol) was condensed with glyoxylic acid. The crude, dark solid (3.0 g, 71%), mp 110–115°, gave an analytical specimen after four recrystallizations from isopropyl ether–acetone as colorless rosettes of microneedles: mp 130–131° dec; ν_{max} 3400 (carboxylic acid), 1700 (carboxylic acid), and 1665 cm^{-1} (ketones).

Anal. Calcd for $C_{22}H_{24}O_8$: C, 63.46; H, 5.81. Found: C, 63.38; H, 5.84.

α-Hydroxy-β-(2-naphthoyl)propionic Acid (15a) and β-Naphthoylacrylic Acid (16a).—To a cooled solution of glyoxylic acid (0.04 mol, 26 ml) was added methyl β-naphthyl ketone (3.4 g, 0.02 mol) in 95% ethyl alcohol (25 ml), followed by an aqueous solution of sodium hydroxide (3 g, 0.075 mol in 54 ml of water). A mixture of 95% ethyl alcohol (50 ml) and water (150 ml) was added to produce homogeneity. The reaction was allowed to proceed for 18 hr at room temperature and at 60° for 10 min. Next the yellow mixture was cooled, diluted with water, and extracted with diethyl ether (two 300-ml portions). The aqueous solution was cooled to 10°, acidified with concentrated hydrochloric acid, and extracted with diethyl ether to provide, after drying and evaporation, a yellow solid (1.5 g). The residue was extracted with hot benzene, and insoluble material (1.1 g, 23%) was crystallized from methanol–water to give colorless crystals of acid 15a, mp 130–132°. Four recrystallizations from the same solvent mixture yielded a pure sample of **α-hydroxy-β-(2-naphthoyl)propionic acid (15a)**: mp 132.5–133.5°; ν_{max} 1730 (carboxylic acid) and 1690 cm^{-1} (ketone).

Anal. Calcd for $C_{14}H_{12}O_4$: C, 68.85; H, 4.95. Found: C, 68.53; H, 5.01.

The benzene-soluble material consisted of a yellow acid (0.4 g, 9%), mp 148–152°. Four recrystallizations from benzene gave an analytical sample of **β-naphthoyl acrylic acid (16a)**: mp 167–168°; $\nu_{max}^{CHCl_3}$ 1710 (carboxylic acid) and 1670 cm^{-1} (conjugated ketone).

Anal. Calcd for $C_{14}H_{10}O_3$: C, 74.32; H, 4.46. Found: C, 74.10; H, 4.56.

Dehydration of α-Hydroxy-β-(2-naphthoyl)propionic Acid (15a).—A solution of α-hydroxy-β-(2-naphthoyl)propionic acid (15a, 0.36 g) in acetic anhydride (10 ml) was heated at 100° for 3 hr with potassium hydrogen sulfate (0.40 g). The solution was cooled, filtered, and diluted with water (10 ml). After 6 hr at room temperature the solution was evaporated to dryness *in vacuo*. The yellow residue was dissolved in saturated aqueous sodium bicarbonate, treated with Norit A, and acidified at 10° with concentrated hydrochloric acid. Upon cooling, the yellow crystalline **β-naphthoylacrylic acid** (16a, 0.12 g, 33%),[46] mp 164–165°, was collected.

Condensation of Methyl Ketones with Glyoxylic Acid. General Procedure A (Ambient).—A rapidly stirred solution of the ketone (0.032 mol) in tetrahydrofuran (250 ml)–methanol (360 ml) was treated successively with aqueous potassium hydroxide (100 ml, 8%) and an aqueous solution of glyoxylic acid (0.076 mol, 27 ml). The pH of the solution was adjusted to 13.65 by gradual addition of 8% aqueous potassium hydroxide. Stirring was continued for 3 days at room temperature. At this point, the yellow mixture was filtered, concentrated to $^1/_4$ volume *in vacuo* at 50°, diluted with water (100 ml), and extracted with diethyl ether. Acidification of the aqueous solution with concentrated hydrochloric acid and extraction with chloroform provided the acidic product. Washing the chloroform solution with saturated aqueous sodium bicarbonate solution removed the acidic components and left in the chloroform neutral products whose infrared spectra indicated presence of a lactone (1780 cm^{-1}). The neutral material was formed (*ca.* 10% of the product) in all cases investigated but was not further characterized.

With Methyl β-Naphthyl Ketone.—Methyl β-naphthyl ketone (10, 5.4 g, 0.032 mol) was condensed with glyoxylic acid by the general procedure A given above to yield acidic (6.5 g) and neutral products (0.7 g); no starting material was recovered. A portion (3.8 g) of the crude acid was dissolved in methanol (115 ml) and heated at reflux for 3 hr with Amberlite IR-120 (H) (3.8 g).[48] The yellow solution was filtered and concentrated *in vacuo* to a yellow oil, which was dissolved in diethyl ether and washed with saturated aqueous sodium bicarbonate (three 20-ml portions) and water (two 20-ml portions). Removal of solvent gave a mobile yellow oil (2.4 g). Acidification of the sodium bicarbonate wash solution and extraction with diethyl ether gave 0.9 g of recovered acid. Repeating the esterification procedure yielded another portion of ester (0.5 g); the total yield of ester was 2.9 g. A sample of the ester (1 g) was chromatographed on acid-washed alumina (30 g) and afforded three distinct products. Elution with 2:5 ligroin–benzene led to a yellow solid (0.17 g), mp 108–109°. The melting point was raised to 112–112.5° by four recrystallizations from methanol to afford a pure sample (characterized in the sequel) of **methyl *trans*-β-(2-naphthoyl)-acrylate (16b)**:[46] ν_{max} 1720 (methyl ester) and 1665 cm^{-1} (conjugated ketone). Continued elution with benzene gave a colorless, mobile oil (0.57 g): ν_{max}^{neat} 1754 (methyl ester), 1694 (ketone), and 1124 cm^{-1} (methoxyl). Distillation at 120° (0.01 mm) gave an analytical specimen of **methyl α-methoxy-β-(2-naphthoyl)-propionate (17b)**.

Anal. Calcd for $C_{16}H_{16}O_4$: C, 70.56; H, 5.92. Found: C, 70.95; H, 5.74.

Elution with 1:1 benzene-chloroform afforded a colorless oil (0.10 g): ν_{max}^{neat} 3571 (hydroxyl), 1748 (methyl ester), and 1690 cm^{-1} (ketone). Distillation at 170° (0.01 mm) gave an analytical specimen of **methyl α-hydroxy-β-(2-naphthoyl)propionate (15b)**.

Anal. Calcd for $C_{15}H_{14}O_4$: C, 69.75; H, 5.46. Found: C, 69.46; H, 5.46.

When the preceding reaction sequence was repeated using 2,4-dimethylacetophenone or 2,5-dimethoxyacetophenone in place of methyl β-naphthyl ketone, examination by thin layer chromatography and infrared spectroscopy of the crude products indicated analogous results. In these examples the actual products were not further identified.

With Methyl Cyclopentyl Ketone.—The ketone (10.6 g, 0.095 mol) was condensed with glyoxylic acid by general procedure A to yield neutral (2.0 g of colorless oil) and acidic (10.0 g, 56%) fractions. Continuous (48 hr) diethyl ether extraction of the acidified reaction mixture was used to isolate the yellow, oily acidic product. A portion (3 g) of the acid was treated with ethereal diazomethane at 0°. Excess diazomethane was destroyed with glacial acetic acid and solvent was removed *in vacuo*. The resulting yellow oil (3.0 g) was chromatographed on a column of acid-washed alumina (90 g) and **methyl α-hydroxy-β-cyclopentylcarbonylpropionate (19)** was eluted by benzene–chloroform mixtures as a colorless oil (2.7 g, 52%). Distillation at 100° (0.1 mm) afforded an analytical specimen: ν_{max} 3546 (hydroxyl), 1740 (methyl ester), and 1709 cm^{-1} (ketone).

Anal. Calcd for $C_{10}H_{16}O_4$: C, 59.98; H, 8.06. Found: C, 59.63; H, 7.96.

With Pinonic Acid.—Pinonic acid[49] (18, 5.8 g, 0.032 mol) in tetrahydrofuran (250 ml)–methanol (360 ml) was condensed with glyoxylic acid, employing general procedure A, to give a dark, oily, acidic product (8.0 g), which was isolated by continuous extraction using diethyl ether. A portion (4.0 g) of the acid was dissolved in diethyl ether containing some methanol and treated with ethereal diazomethane. Excess diazomethane was destroyed by adding a few drops of glacial acetic acid. Removal of solvent *in vacuo* furnished a dark-colored oil (4.0 g), which was chromatographed on a column of acid-washed alumina (120 g). The product (3.3 g, 72%), **methyl α-hydroxy-β-pinonoyl propionate (20)**, was eluted by benzene–chloroform mixtures as a pale yellow oil which was purified by distillation: bp 170° (0.1 mm); ν_{max}^{neat} 3400 (hydroxyl), 1740 (methyl esters), and 1705 cm^{-1} (ketone).

Anal. Calcd for $C_{14}H_{22}O_5$: C, 58.73; H, 7.75. Found: C, 58.35; H, 7.65.

General Procedure B (Heating).—To a stirred solution of the ketone (0.013 mol) in tetrahydrofuran (100 ml)–methanol (150 ml) was added potassium hydroxide solution (30 ml, 8%), followed by aqueous glyoxylic acid (0.03 mole, 11 ml). The pH was adjusted as specified by addition of aqueous potassium hydroxide (8%) and the mixture was stirred for 60 min at room

(48) P. J. Mill and W. R. C. Crimmin, *Biochem. Biophys. Acta*, **23**, 432 (1957).

(49) J. B. Lewis and G. W. Hedrick, *J. Org. Chem.*, **24**, 1870 (1959). We wish to thank Dr. Hedrick for a generous supply of pinonic acid.

temperature followed by 12 hr at reflux. The acidic product(s) was isolated as summarized in general procedure A.

With Methyl β-Naphthyl Ketone.—Condensing methyl β-naphthyl ketone (10, 2.2 g, 0.013 mol) with glyoxylic acid using general procedure B (pH 13.20) led to 2.7 g of crude acidic product. A portion (0.5 g) of this material was dissolved in methanol (8 ml) and treated with 2,2-dimethoxypropane (Dow Chemical Co.) followed by concentrated hydrochloric acid (4 drops).[50] The yellow solution was heated to 50° and treated with four 1-ml portions of 2,2-dimethoxypropane at 1-hr intervals. The temperature was maintained at 50° for 17 hr, after which most of the solvent was removed *in vacuo* and water (50 ml) was added. Following extraction of the turbid aqueous mixture with chloroform and washing of the extract with saturated aqueous sodium bicarbonate and water, drying, and evaporating, a yellow oil (0.6 g) was obtained. Chromatography on a column of neutral alumina (18 g) and eluting with a series of hexane–benzene mixtures afforded a pale yellow solid (0.30 g, 57%): mp 108–109°; ν_{max} 1720 (methyl ester), 1665 (conjugated ketone), and 1630 cm^{-1} (double bond). Four recrystallizations from methanol gave an analytical specimen of **methyl *trans*-β-(2-naphthoyl)acrylate (15b)** as pale yellow needles: mp 112–112.5° (lit.[51] mp 112–113°); pmr (CCl_4) δ 3.74 (singlet, 3 methyl protons) and 6.61 and 6.88 (doublet, J = 15 cps, 1 proton, the second olefin proton was obscured by the aromatic proton region).

Anal. Calcd for $C_{15}H_{12}O_3$: C, 74.99; H, 5.04. Found: C, 74.64; H, 4.84.

With 2,4-Dimethylacetophenone.—A 1.9-g (0.013 mol) sample of 2,4-dimethylacetophenone was condensed with glyoxylic acid by general procedure B at pH 13.20 to give 2.2 g of acidic product. A portion (0.5 g) of the acidic fraction was esterified using methanol and 2,2-dimethoxypropane as described above for the preparation of methyl-β-(2-naphthoyl)-acrylate and provided a dark yellow oil (0.60 g). Column chromatography on neutral alumina (15 g) and elution with hexane yielded a yellow oil (0.30 g, 56%), ν_{max}^{neat} 1725 (methyl ester), 1670 (conjugated ketone), and 1630 cm^{-1} (double bond). The oil crystallized after 24 hr at 0°. Four recrystallizations from 2-propanol gave a yellow, crystalline, analytical specimen of **methyl β-(2,4-dimethylbenzoyl)acrylate (21)**, mp 50–50.2°.

Anal. Calcd for $C_{13}H_{14}O_3$: C, 71.54; H, 6.46. Found: C, 71.35; H, 6.44.

With 2,5-Dimethoxyacetophenone.—The acetophenone (2.3 g, 0.013 mol) was condensed with glyoxylic acid by general procedure B at pH 13.65 and the crude acidic product (2.9 g) was esterified by treatment for 3 hr with Amberlite IR-120 (H) (2.9 g) in boiling methanol (75 ml). The yellow solution was filtered and the solvent was removed *in vacuo* to yield a dark, oily residue which was dissolved in diethyl ether. The ethereal solution was washed with saturated aqueous sodium bicarbonate and water, dried, and evaporated to furnish a yellow oil (1.7 g). This residue was chromatographed on a column of acid-washed alumina (40 g). Elution with 1:1 hexane–benzene gave a yellow oil (1.0 g, 59%), which crystallized from 2-propanol in matted, yellow needles (0.9 g), mp 65–68°. Four recrystallizations from 2-propanol yielded an analytical specimen of **methyl β-(2,5-dimethoxybenzoyl)acrylate (22)**: mp 73–73.5° (lit.[52] mp 65°); ν_{max}^{Nujol} 1727 (methyl ester), 1672 (conjugated ketone), and 1636 cm^{-1} (double bond).

Anal. Calcd for $C_{13}H_{14}O_5$: C, 62.40; H, 5.64. Found: C, 62.65; H, 5.80.

Further elution of the column with chloroform gave a dark oil (0.15 g). Distillation at 170° (0.01 mm) afforded **methyl α-hydroxy-β-(2,5-dimethoxybenzoyl)propionate (23)** as a pale yellow oil, ν_{max}^{neat} 3570 (hydroxyl), 1754 (methyl ester), and 1677 cm^{-1} (ketone).

Anal. Calcd for $C_{13}H_{16}O_6$: C, 58.20; H, 6.01. Found: C, 58.53; H, 6.59.

Methylation of Methyl α-Hydroxy-β-(2-naphthoyl)propionate (15b).—To a solution of methyl α-hydroxy-β-(2-naphthoyl)-propionate (15b, 0.5 g) in diethyl ether (5 ml) at −10° was added 1 drop of boron trifluoride etherate followed dropwise by an ethereal solution of diazomethane from 1.2 g of nitrosomethylurea during 5 min.[53] Cooling was maintained until the yellow color had disappeared (15 min). A gelatinous precipitate was removed by filtration and the filtrate was washed with saturated aqueous sodium bicarbonate, dried, and concentrated to a colorless oil (0.2 g). The residue was chromatographed on acid-washed alumina (15 g), and **methyl α-methoxy-β-(2-naphthoyl)-propionate (15b, 0.1 g, 18%)** was obtained, eluted by benzene, as a colorless oil.

Alternate Synthesis of Methyl *trans*-β-(2-Naphthoyl)acrylate (16b).—To a stirred solution of ω-bromo-2-acetonaphthone (2.4 g, 0.01 mol) in dry tetrahydrofuran (20 ml) was added in one portion a warm solution of carbomethoxymethylenetriphenylphosphorane[54] (6.7 g, 0.02 mol) in dry tetrahydrofuran (30 ml). After 24 hr at room temperature the solution was filtered to remove carbomethoxymethyltriphenylphosphonium bromide (2.6 g, 64%). The filtrate was evaporated to a dark oil, which was diluted with dry benzene and treated with methyl iodide for 2 hr at 5°. Filtration and evaporation yielded a dark residue which was chromatographed (column) on acid-washed alumina (30 g). The hexane–benzene fractions yielded a yellow solid 16,[46] which recrystallized from methanol as pale yellow plates (0.91 g, 38%), mp 110–111°.

Methyl *cis*-β-(2-Naphthoyl)acrylate (16b).—Methyl *trans*-β-(2-naphthoyl)acrylate (16a, 0.20 g) in benzene (10 ml) was irradiated with a sun lamp (GE 110–125 V) at a distance of 4 ft for 48 hr, at which time tlc examination showed almost complete conversion into the more polar *cis* isomer. Evaporation of solvent gave an orange oil which slowly crystallized on trituration with hexane. The crude product was heated with hexane and the hot solution was decanted from an oily by-product. The product, which crystallized upon cooling, was recrystallized three times from hexane to give cream-colored crystal clusters (0.06 g): mp 92–95°; pmr (CCl_4) δ 3.41 (singlet, 3 methyl protons) and 5.94, 6.13, 6.58, and 6.77 (quartet, J = 11 cps, 2 protons, *cis* isolated double bond).

3β-Triphenylmethoxy-20-oxo-5α-pregnane (24c).—A solution of 3β-hydroxy-20-oxo-5α-pregnane (24a, 3.6 g, 11 mmol) and triphenylmethyl bromide (5.4 g, 17 mmol) in dry pyridine (100 ml) was heated at 100° for 12 hr. The yellow reaction mixture was cooled and poured onto ice, and the resulting yellow precipitate was collected by extraction with chloroform. The chloroform extract was washed twice with water, dried, and evaporated to yield a yellow gum which solidified on trituration with diethyl ether. Filtration provided a cream-colored solid (3.87 g), mp 216–220°. Recrystallization from chloroform–methanol gave cream-colored needle clusters in two crops of 2.2 g, mp 222–225°, and 1.3 g, mp 218–223°. Recrystallization of the second crop from the same solvent mixture gave 1.19 g, mp 223–227°. Yield (of almost pure material) was 3.39 g (53%). An analytical specimen was prepared by three recrystallizations from the same solvent mixture: mp 227–230°; ν_{max} 1695 (ketone) and 1030–1050 cm^{-1} (ether); $[\alpha]^{22}D$ +39.2° (c 1.46).

Anal. Calcd for $C_{40}H_{48}O_2$: C, 85.66; H, 8.63. Found: C, 85.11; H, 8.26.

3β-Triphenylmethoxy-20-oxo-5-pregnene (7c).—Treatment of 3β-hydroxy-20-oxo-5-pregnene (7a, 5.0 g, 16 mmol) with triphenylmethyl bromide in pyridine was performed exactly as described above for the 5α analog 24c to yield a brown, glasslike crude product (11.4 g). A solution of the residue in 5:1 hexane–benzene was chromatographed on basic alumina (250 g). The benzene–hexane eluate gave a colorless solid which crystallized from chloroform–methanol as needles (4.7 g, 53%), mp 180–185°. An analytical specimen was prepared by trituration with boiling methanol followed by six recrystallizations of the insoluble material from chloroform–methanol: mp 188–191°; $[\alpha]^{22}D$ +23.2° (c 1.29); ν_{max} 1690 (ketone) and 1040 cm^{-1} (ether).

Anal. Calcd for $C_{40}H_{46}O_2$: C, 85.98; H, 8.30. Found: C, 85.49; H, 8.05.

3β-Triphenylmethoxy-20-oxo-5,16-pregnadiene (25b).—A 5.0-g (16 mmol) sample of 3β-hydroxy-20-oxo-5,16-pregnadiene (25a) was treated with triphenylmethyl bromide in hot pyridine for 24 hr as described above for 24c. The product was a dark, viscous oil (10 g). Column chromatography on basic alumina (250 g) and elution with hexane–benzene mixtures gave a colorless solid which crystallized from chloroform–methanol as glistening needles (4.5 g, 51%), mp 190–194°. Three recrystal-

(50) N. B. Lorette and J. H. Brown, Jr., *J. Org. Chem.*, **24**, 261 (1959).

(51) R. H. Martin and P. Stoffyn, *Bull. Soc. Chim. Belg.*, **59**, 83 (1950); *Chem. Abstr.*, **45**, 594 (1951).

(52) K. P. Dave and K. S. Nargund, *J. Univ. Bombay*, **7**, Part 3, 191 (1938); *Chem. Abstr.*, **33**, 3779 (1939).

(53) M. Neeman, M. C. Caserio, J. D. Roberts, and W. S. Johnson, *Tetrahedron*, **6**, 36 (1959).

(54) O. Isler, H. Gutmann, M. Montavon, R. Rüegg, G. Ryser, and P. Zeller, *Helv. Chim. Acta*, **40**, 1242 (1957).

lizations from acetone gave an analytical sample: mp 195–197°; $[\alpha]^{22}D$ −21.4° (*c* 1.215); ν_{max} 1665 (conjugated ketone) and 1040 cm^{-1} (ether).

Anal. Calcd for $C_{40}H_{44}O_2$: C, 86.27; H, 7.97. Found: C, 86.22; H, 7.98.

3β-Methoxymethyloxy-20-oxo-5-pregnene (7d).—A solution of 3β-hydroxy-20-oxo-5-pregnene (7a, 7.5 g, 24 mmol) in refluxing chloromethyl methyl ether (75 ml, Eastman) containing suspended Drierite (20 g) was treated in portions with freshly prepared, dry silver oxide (17 g) during 90 min. Heating was continued for 4 hr. The solvent was reduced in volume and filtered, and the inorganic residue was washed well with chloroform. The residue (9.0 g) obtained upon removal of solvent *in vacuo* was chromatographed on basic alumina (200 g). Elution with benzene gave a colorless solid (1.5 g, 18%), mp 95–100°. Four recrystallizations from methanol gave a pure specimen as colorless microneedles: mp 103–104°; $[\alpha]^{22}D$ 0°; ν_{max} 1700 (ketone), 1150, 1100, and 1040 cm^{-1} (ether).

Anal. Calcd for $C_{23}H_{36}O_3$: C, 76.64; H, 10.07. Found: C, 77.23; H, 9.94.

Elution of the column with chloroform yielded unreacted 3β-hydroxy-20-oxo-5-pregnene (5.5 g).

Condensation of 3β-Hydroxy-20-oxo-5-pregnene (7a) with Glyoxylic Acid at Readings of pH 13.25–13.48. Experiment A. pH 13.25.—A vigorously stirred solution of 3β-hydroxy-20-oxo-5-pregnene (7a, 2.0 g, 6.3 mmol) in tetrahydrofuran (50 ml)–methanol (75 ml) was treated successively with potassium hydroxide solution (8% aqueous, 20 ml), and a solution of glyoxylic acid (23 mmol, 6 ml) prepared as described above. Potassium hydroxide (8% aqueous) was then gradually added until the pH meter scale reading was 13.25. Stirring was continued for 3 days at room temperature, and the solution was filtered, concentrated *in vacuo* to $^1/_5$ volume, and diluted with water (100 ml). Extracting the basic mixture with chloroform followed by washing with water, drying, and concentration furnished unreacted 3β-hydroxy-20-oxo-5-pregnene (7a, 1.3 g).[46] Acidification of the aqueous solution with concentrated hydrochloric acid gave a gelatinous, acidic product which was collected by chloroform extraction to yield a colorless, amorphous, acidic product (0.63 g, 25%): mp 160–170° dec; ν_{max} 3400 (broad, acid) and 1750–1690 cm^{-1} (carboxylic acid and ketone).

Experiment B. pH 13.48.—Experiment A was repeated with ketone 7a (3 g, 9.5 mmol) exactly as above except that the pH reading was adjusted to 13.48. The neutral extract gave starting material (1.3 g), and the acidic fraction yielded a colorless, amorphous solid (1.6 g, 45%), mp 155–175° dec.

Methyl 3β,23-Dihydroxy-20-oxo-21-nor-5-cholenate (26b).—Condensation of 3β-hydroxy-20-oxo-5-pregnene (7a, 20 g, 63.4 mmol) with glyoxylic acid was carried out at pH 13.48 exactly as described above (experiment B) to yield starting material (8.5 g) and amorphous acid (12.3 g, 50%), mp 155–175° dec. A portion (11 g) of the acidic material, dissolved in methanol, was treated with ethereal diazomethane at 0°. Excess diazomethane was decomposed immediately with a few drops of glacial acetic acid, and the solvent was removed *in vacuo*. The yellow, viscous oil (11.5 g) was chromatographed on acid-washed alumina (250 g). A pale yellow solid (4 g) was eluted by 1:1 benzene–chloroform; purification of this material will be described below (see 26a). Elution with chloroform furnished a pale yellow solid (7 g, 30%), mp 154–164°, which was homogeneous as evidenced by thin layer chromatography. Four recrystallizations from isopropyl ether–methanol afforded an analytical sample of diol 26b as colorless plates: mp 170–174°; $[\alpha]^{22}D$ +9.0°; ν_{max} 3400 (hydroxyl), 1740 (methyl ester), and 1700 cm^{-1} (ketone).

Anal. Calcd for $C_{24}H_{36}O_5$: C, 71.25; H, 8.97. Found: C, 71.18; H, 8.80.

A sample (2.3 g) of the diol was acetylated, and the crude diacetate (26c, 2.6 g) was decolorized by two treatments with Norit-A in methanol. Recrystallization from aqueous methanol gave the diacetate (2.2 g, 79%) as colorless plates, mp 105–107°. An analytical specimen of methyl 3β,23-diacetoxy-20-oxo-21-nor-5-cholenate (26c) was prepared by five recrystallizations from aqueous methanol: mp 105–108°; $[\alpha]^{22}D$ +23.2° (*c* 1.242); ν_{max}^{Nujol} 1754 (sh, methyl ester), 1738 (acetates), 1709 (ketone) and 1250 cm^{-1} (acetates).

Anal. Calcd for $C_{28}H_{40}O_7$: C, 68.82; H, 8.25. Found: C, 68.90; H, 7.96.

Methyl 3β,23-Ditetrahydropyranyloxy-20-oxo-21-nor-5-cholenate (26d).—To a magnetically stirred suspension of 3β,23-dihydroxy-20-oxo-21-nor-5-cholenate (26b, 1.25 g) in dry benzene (40 ml) and dihydropyran (7.5 ml, distilled from sodium) was added *p*-toluenesulfonic acid monohydrate (75 mg). After 2 min the solution became homogeneous, and stirring was continued at room temperature for 30 min.[55] The pale yellow solution was washed with sodium hydroxide solution (1% in 1:1 methanol-water) and water. Removal of solvent gave a yellow oil (2.0 g) which was chromatographed on neutral alumina (30 g). Elution with 1:1 benzene–hexane gave a colorless oil (1.25 g, 70%) which solidified, mp 70–75° (vacuum dried) upon trituration with cold (ice-bath) methanol. An analytical sample was prepared by three recrystallizations from methanol, followed by two from aqueous acetone: mp 70–78°; ν_{max} 1750 (methyl ester), 1705 (ketone), and 1030 cm^{-1} (split, ethers).

Anal. Calcd for $C_{34}H_{52}O_7$: C, 71.29; H, 9.15. Found: C, 71.53; H, 9.10.

Methyl 3β-Acetoxy-23-methoxy-20-oxo-21-nor-5-cholenate (26a).—A portion (1.5 g) of the material (4 g) eluted in 1:1 benzene–chloroform (see 26a above) during isolation of methyl 3β,23-dihydroxy-20-oxo-21-nor-5-cholenate (26a) was acetylated. The oily acetate (1.2 g) was chromatographed on acid-washed alumina (30 g). A 0.3-g quantity of 3β-acetoxy-20-oxo-5-pregnene (7b) was eluted by 1:1 benzene–hexane. Elution with benzene gave a colorless solid (0.7 g), mp 102–103°. Three recrystallizations from diethyl ether–hexane gave a pure specimen as colorless plates: mp 104–105°; $[\alpha]^{20}D$ +20.5° (*c* 2.879); ν_{max}^{Nujol} 1754 (methyl ester), 1733 (acetate), 1709 (ketone), 1250 (acetate), and 1136 cm^{-1} (methoxy); pmr δ 3.24 (3 methoxy protons) and 3.58 (3 methyl ester protons).

Anal. Calcd for $C_{27}H_{40}O_6$: C, 70.40; H, 8.76. Found: C, 70.80; H, 8.68.

Condensation between 3β-Hydroxy-20-oxo-5-pregnene (7a) and Glyoxylic Acid at pH 13.65. Method A. Room Temperature.—Reaction between 3β-hydroxy-20-oxo-5-pregnene (7a, 20 g, 63.4 mmol) and glyoxylic acid at pH 13.65 was accomplished as described above (*cf.* 26b). After 28 hr, half of the reaction mixture was taken for isolation studies and the other half was allowed to proceed for an additional 48 hr at room temperature. Isolation work with the first half was complicated by emulsion formation (during extraction of the basic solution with chloroform), and caused inefficient separation into neutral and acidic fractions. The neutral extract furnished 3β-hydroxy-20-oxo-5-pregnene (0.6 g, 7a), and the acidic portion was obtained as a pale yellow foam (10 g). The acid (10 g) was dissolved in 10:1 chloroform–methanol and treated with ethereal diazomethane at ice-bath temperature. Excess diazomethane was destroyed at once with a few drops of glacial acetic acid. The solution was concentrated to a yellow oil (11 g). Acetylation gave a yellow, viscous oil (11.5 g) which was chromatographed on acid-washed alumina (300 g). Elution with 1:1 hexane–benzene gave **3β-acetoxy-20-oxo-5α-pregnane (7b, 4.3 g)**, and benzene gave a mixture of two components, separable by crystallization from methanol, to give first the less soluble **methyl 3β-acetoxy-20-oxo-21-nor-5-*trans*-22-choladienate** (27, 0.60 g, 5%), mp 153–156°. Three recrystallizations from methanol gave long yellow needles: mp 157–158.5°; $[\alpha]^{20}D$ +37.5° (*c* 1.27); ν_{max} 1735 (methyl ester), 1730 (acetate), 1690 (conjugated ketone), and 1628 cm^{-1} (m, conjugated double band).

Anal. Calcd for $C_{26}H_{36}O_5$: C, 72.86; H, 8.47. Found: C, 73.34; H, 8.43.

The second component eluted by benzene was identical[46] with **methyl 3β-acetoxy-23-methoxy-20-oxo-5-cholenate** (26a, 1.6 g, 11%), mp 102–104°. Further elution of the column with 2:1 benzene–chloroform gave **methyl 3β,23-diacetoxy-20-oxo-21-nor-5-cholenate** (26c, 2.3 g, 15%),[46] mp 96–99°, and a pale yellow, viscous oil (1.0 g). An infrared spectrum suggested that the oil represented **pyrazoline 28**: ν_{max} 1745 (methyl ester), 1701 (ketone), and 1577 cm^{-1} (pyrazoline). Continued elution with 10:1 and 4:1 benzene–chloroform gave mixtures (shown by thin layer chromatography) of the 23-acetoxy (26b) and 23-methoxy (26d) esters.

After 72 hr the second half of the reaction product was methylated and acetylated in the same way to give 3β-hydroxy-20-oxo-5-pregnene (7a, 0.8 g) as the initial neutral fraction and a mixture of methyl esters (9.0 g) from the initial acid fraction. Chromatography of the methyl ester mixture as before gave 3β-

(55) Normal reaction time (3 hr) for tetrahydropyranyl ether formation produced a mixture of products. After several experiments over various periods, the 30-min procedure was found most satisfactory.

acetoxy-20-oxo-5-pregnene (**7b**, 1.6 g), methyl 3β-acetoxy-20-oxo-21-nor-5,22-choladienate (**27**, 1.0 g, 8%), methyl 3β-acetoxy-23-methoxy-20-oxo-21-nor-5-cholenate (**26a**, 2.0 g, 14%), methyl 3β,23-diacetoxy-20-oxo-21-nor-5-cholenate (**26c**, 3.0 g, 19%), and pyrazoline **28** (0.4 g).

Method B. Reflux Temperature.—Here, 3β-hydroxy-20-oxo-5-pregnene (**7a**, 20 g, 63.4 mmol) was condensed with glyoxylic acid as described above at pH 13.65 except that the reaction was heated at reflux temperature (60°) for 8 hr. Despite an inefficient separation because of emulsification, 2.4 g of unreacted starting material and 19.5 g of acidic material (as a pale yellow foam) were isolated. Methylation (see method A) and acetylation gave a dark oil (20 g) which was chromatographed on acid-washed alumina (500 g). Elution with the same solvents as described in the preceding experiment gave 3β-acetoxy-20-oxo-5-pregnene (**7b**, 3.56 g), methyl 3β-acetoxy-20-oxo-21-nor-5,22-choladienate (**27**, 2.1 g, 8%), methyl 3β-acetoxy-23-methoxy-20-oxo-21-nor-5-cholenate (**26a**, 0.3 g, 1%), methyl 3β-23-diacetoxy-20-oxo-21-nor-5-cholenate (**26c**, 2.8 g, 9%), and pyrazoline **28** (5.5 g).

In another experiment[56] a portion (17 g) of the acidic product prepared by method B (reflux, 8 hr) in methanol (170 ml) was treated with 2,2-dimethoxypropane (17 ml) and warmed to 50° with concentrated hydrochloric acid (5 ml). After 1 hr, more dimethoxypropane (17 ml) was added, and this operation was repeated twice more at hourly intervals. One day later the brown solution was filtered and concentrated *in vacuo*. Addition to water and extraction with chloroform furnished the crude product. Acetylation led to a pale yellow solid (17 g). Chromatography on acid-washed alumina (450 g) and elution with benzene gave methyl 3β-acetoxy-20-oxo-21-nor-5-*trans*-22-choladienate (**27**, 5.0 g),[46] mp 154–155°. In this case the other products were not isolated.

Condensation between 3β-Acetoxy-20-oxo-5α-pregnane (24a) and Glyoxylic Acid.—Using method A (see above with ketone **7a**, pH 13.65), 3β-acetoxy-20-oxo-5α-pregnane (**24a**, 10 g, 28 mmol) was condensed with glyoxylic acid. A period of 3 days at room temperature afforded starting material (5.1 g) after reacetylation and acidic product (4.82 g), mp 198–205° dec, collected by filtration. A sample of the acid was recrystallized five times from aqueous ethyl alcohol to give the analytical specimen of **3β,23-dihydroxy-20-oxo-21-nor-5α-cholanic acid (29a)** as colorless plates: mp 227–229° dec; ν_{max} 3350 (hydroxyl), 2700–2300 (w, carboxyl), 1730 (carboxyl), and 1698 cm^{-1} (ketone).

Anal. Calcd for $C_{23}H_{36}O_5$: C, 70.37; H, 9.24; O, 20.38. Found: C, 70.80; H, 9.00; O, 20.20.

A solution of the crude acid (mp 198–205° dec, 4.8 g) in 1:1 chloroform–methanol (200 ml) was converted into the methyl ester with ethereal diazomethane. Excess reagent was quickly destroyed with acetic acid, and the solution was washed with saturated sodium bicarbonate solution followed by water. Removal of solvent furnished a yellow, oily residue. The crude ester was chromatographed on acid-washed alumina (140 g). Elution with 7:3 benzene–chloroform gave a yellow oil (1.1 g) which slowly solidified and showed two closely positioned spots on a thin layer chromatogram. Elution with 1:1 benzene–chloroform led to a colorless solid (2.0 g), mp 147–157°. Recrystallization from diethyl ether containing a trace of methanol gave colorless needles (1.0 g), mp 165–167°. Three recrystallizations from the same solvent gave **methyl 3β,23-dihydroxy-20-oxo-21-nor-5α-cholanate (29b)** as needles: mp 177–179°; $[\alpha]^{20}D$ +91.8° (*c* 1.381); ν_{max} 3200, 3100 (hydroxyls), 1739 (methyl ester), and 1697 cm^{-1} (ketone).

Anal. Calcd for $C_{24}H_{38}O_5$: C, 70.90; H, 9.42; O, 19.68. Found: C, 70.84; H, 9.24; O, 20.16.

Acetylation of a sample of the dihydroxymethyl ester (0.09 g), mp 174–176°, gave diacetate **29c** (0.12 g), mp 109–113°, and successive recrystallization from hexane and aqueous ethyl alcohol gave the analytical specimen of **methyl 3β,23-diacetoxy-20-oxo-21-nor-5α-cholanate (29c)** as colorless plates: mp 113–114.5°; $[\alpha]^{21}D$ +71.1° (*c* 0.82); ν_{max} 1725–1750 (acetates and methyl ester) and 1695 cm^{-1} (ketone).

Anal. Calcd for $C_{28}H_{42}O_7$: C, 68.54; H, 8.63; O, 22.83. Found: C, 68.52; H, 8.79; O, 22.92.

Conversion of Methyl 3β,23-Dihydroxy-20-oxo-21-nor-5α-cholanate (29b) into 3β-Hydroxy-20-oxo-5α-pregnane (24a).—A solution of methyl 3β,23-dihydroxy-20-oxo-21-nor-5α-cholanate (**29b**, 0.1 g) in methanol (20 ml) containing potassium hydroxide (1 g) was heated at reflux for 2 hr. Concentration *in vacuo* to a small volume, followed by dilution with water, three extractions with chloroform, and evaporation of solvent gave a colorless, crystalline solid (25 mg) identical[46] with **3β-hydroxy-20-oxo-5α-pregnane (24a)**. Acidifying the alkaline solution provided a 0.072-g acid fraction.

Registry No.—Glyoxylic acid, 298-12-4; **7c**, 23328-04-3; **7d**, 23328-05-4; **11a**, 23349-18-0; **11b**, 23389-68-6; **11c**, 23349-19-1; **14**, 23349-20-4; **15a**, 23359-85-5; **15b**, 23349-21-5; **16a**, 23328-06-5; **16b**, 23328-07-6; **17b**, 23349-22-6; **19**, 23349-23-7; **20**, 23349-24-8; **21**, 23349-25-9; **22**, 23349-26-0; **23**, 23349-27-1; **24c**, 23328-08-7; **25b**, 23328-09-8; **26a**, 23328-10-1; **26b**, 23328-11-2; **26c**, 23328-12-3; **26d**, 23328-13-4; **27**, 23330-45-2; **28**, 23328-15-6; **29a**, 23328-16-7; **29b**, 23328-17-8; **29c**, 23328-18-9.

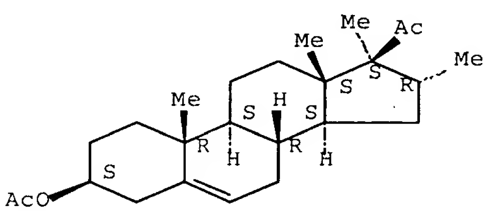
(56) Performed by Dr. A. K. Das Gupta.

Compound (V)

CAS Registry Record:

RN 13116-52-4 REGISTRY
ED Entered STN: 16 Nov 1984
CN Pregn-5-en-20-one, 3-(acetyloxy)-16,17-dimethyl-, (3β,16α)- (9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN Pregn-5-en-20-one, 3β-hydroxy-16α,17-dimethyl-, acetate (7CI, 8CI)
OTHER NAMES:
CN 16α,17α-Dimethylpregnenolone acetate
FS STEREOSEARCH
MF C25 H38 O3
LC STN Files: BEILSTEIN*, CA, CAOLD, CAPLUS, CASREACT, CHEMCATS, USPATFULL
(*File contains numerically searchable property data)

Absolute stereochemistry.



8 REFERENCES IN FILE CA (1907 TO DATE)
8 REFERENCES IN FILE CAPLUS (1907 TO DATE)
1 REFERENCES IN FILE CAOLD (PRIOR TO 1967)

Oldest Lit Refs in Chem Abs:

L16 ANSWER 1 OF 1 CAOLD COPYRIGHT 2006 ACS on STN
AN CA59:10161f CAOLD
TI gas chromatography of selected pregnenes and pregnanes
AU Nelson, James P.
IT 982-06-9 1769-67-1 1808-63-5 1863-39-4 2193-00-2 2601-07-2
2857-83-2 5456-44-0 6661-94-5 13116-52-4 14148-09-5
14231-06-2 14279-42-6 16394-71-1 40148-10-5 55334-07-1 55530-52-4
56630-86-5 56784-22-6 56784-24-8 56792-49-5 101399-58-0 101635-49-8

L18 ANSWER 4 OF 6 HCA COPYRIGHT 2006 ACS on STN
AN 78:148118 HCA Full-text
TI Preparation of formates of isomeric pregnane 5,6-bromohydrins
AU Bayunova, V. I.; Akalaev, A. N.; Pakhomov, V. P.; Grinenko, G. S.
CS Vses. Nauchno-Issled. Khim.-Farm. Inst., Moscow, USSR
SO Khimiya Prirodnykh Soedinenii (1973), 9(1), 39-45
CODEN: KPSUAR; ISSN: 0023-1150
DT Journal
LA Russian
GI For diagram(s), see printed CA Issue.
AB Pregnenolone acetate was treated with aqueous HOBr in DMF to yield bromopregnanediol formates I and II and pregnanetriol acetate III (R = R1 = H). Similarly, 16,17-dimethyl-pregnenolone acetate (IV) gave I and II (R = R1 = Me); and 16,17-

epoxypregnenolone acetate yielded I, II (RR1 = O) and 5α-bromo-16,17-epoxy-6β-hydroxypregnenolone 3-acetate. IV was treated with aqueous HOBr in EtOAc to give the bromohydroxy- pregnanones V and VI (R = R1 = Me). The 5,6-bromohydrin compds. yielded 5,6-epoxy derivs. when treated with KOAc or Kk2CO3.
CC 32-5 (Steroids)
IT 1778-02-5 13116-52-4 34209-81-9
RL: RCT (Reactant); RACT (Reactant or reagent)
(reaction of, with hypobromous acid and DMF)

L18 ANSWER 5 OF 6 HCA COPYRIGHT 2006 ACS on STN
AN 67:44004 HCA

Full Text

TI Synthesis of 17-bromo-16α-methylprogesterones
AU Reimann, Hans; Sarre, Olga Z.
CS Schering Corp., Bloomfield, NJ, USA
SO Journal of Organic Chemistry (1967), 32(7), 2321-4
CODEN: JOCEAH; ISSN: 0022-3263
DT Journal
LA English
GI For diagram(s), see printed CA Issue.
AB In situ bromination of 16α-methylpregnane 20-magnesium enolates gives a mixture of the 17α-bromo- and 17β-bromo-20-oxo derivs. Utilizing this reaction, 17α-bromo-16α-methylprogesterone (II) and 17β-bromo-16α-methyl-17-isoprogesterone (I) were prepared from 16-dehydropregnenolone acetate (III). 21 references.
CC 32 (Steroids)
IT 982-06-9P 983-23-3P 13116-47-7P 13116-48-8P 13116-50-2P
13116-51-3P 13116-52-4P 13143-64-1P
RL: SPN (Synthetic preparation); PREP (Preparation)
(preparation of)

L18 ANSWER 6 OF 6 HCA COPYRIGHT 2006 ACS on STN
AN 59:55330 HCA Full-text
OREF 59:10161f-h,10162a
TI Gas chromatography of selected pregnenes and pregnanes
AU Nelson, J. P.
CS Gen. Mills Res. Labs., Minneapolis, MN
SO Journal of Gas Chromatography (1963), 1(3), 27-9
CODEN: JGCRAY; ISSN: 0096-2686
DT Journal
LA Unavailable
AB Relative retention times on SE-30 silicone gum and on QF-1-0065 fluorinated silicone both at 221° are given: (SE-30, QF-1-0065) for 5β-16-pregnene-3β-ol-20-one (0.55, 2.17) and acetate (0.75, 3.78), 16-dehydropregnenolone (0.58, 2.42) and acetate (0.86, 4.04), pregnenolone (0.63, 2.76) and acetate (0.94, 4.81), 16α-methylpregnenolone (0.64, 2.64) and acetate (0.93, 4.16), 16α,17α-epoxypregnenolone (0.66, 2.96), 16-methyl-16- dehydropregnenolone (0.74, 3.10) and acetate (1.12, 5.31), 5β-pregnane-3β,17α-diol-20-one (0.86, 3.45), 17α-hydroxypregnenolone (0.88, 4.13) and acetate (1.25, 6.93), 5α,6α-epoxy-5α-pregnan-3β-ol-20-one (0.92, 5.88) and acetate (1.41, 11.4), 6β,16α-dimethyl-5α-pregnane- 3β,5α-diol-20-one (1.29, 6.41) and acetate (1.29, 10.8), 6β-methyl-5αpregnane-3β,5α-diol-20-one (1.31, 7.13), 16α,17α-dimethylpregnenolone acetate (1.00, 5.10), 6,16α-dimethylpregnenenolone acetate (1.07, 4.20), 16β-methylpregnenenolone acetate (108, 4.40), 5β,6β-epoxypregnan-3β-ol-20-one 3-acetate (1.23, decompose), 5α,6α-epoxy-16-pregnen-3β-ol-20-one 3-acetate (1.32, 10.5), 5α-pregnane-3β,17α-diol-20-one 3-acetate (1.32,

7.42), 5α,6α: 16α, 17α-diepoxy-5α-pregnan- 3β-ol-20-one-3 acetate (15.0, not eluted), 20,20- ethylenedioxypregnenenolone acetate (1.79, 4.30), 16α-methyl-20,20-ethylenedioxypregnenenolone acetate (1.85, 4.20), 16α-methyl- 5α,6α-epoxy-5α-pregnane-3β,17αdiol-20-one 3-acetate (2.44, not eluted), 5α,6α-epoxy-20,20-ethylenedioxy- 5α-pregnan-3β-ol 3-acetate (2.80, 11.5), 5α,6αepoxy- 16α-methyl-20,20-ethylenedioxy- 5α-pregnan-3β-ol 3-acetate (2.85, 10.7). Relative retention times are given by the formula x = y + a + b + ... + n where x is the time of the substance to be calculated, y that of the parent substance, and a, b, ... n, the contributions of substituent groups as follows (acetates): 16α-methyl (-0.01, -0.65), Δ16 (-0.08, -0.77), 16α,17α-epoxy (0.06, 0.29), 16β-methyl (0.14, -0.41), 17α-hydroxy (0.31, 2.12), 5α,6α-epoxy (0.48, 6.59), 20,29-ethylenedioxy (0.85, -0.51), 6-methyl (0.14, 0.04); (alcohols): 16α-methyl (0.01, -0.12), Δ16 (-0.05, -0.34), 17α-hydroxy (0.25, 1.37), 5α,6α-epoxy (0.29, 3.12). Results are within 1.5% of exptl. values, except in the case of 5α,6α-epoxy-20,20-ethylenedioxy- 5α-pregnan-3β-ol 3-acetate on SE-30 which is 23% in error.

CC 42 (Steroids)

IT 570-53-6, 5β-Pregnan-20-one, 3β,17-dihydroxy- 974-23-2, Pregn-5-en-20-one, 16α,17-epoxy-3β-hydroxy- 982-06-9, Pregna-5,16-dien-20-one, 3β-hydroxy-16-methyl-, acetate 1162-53-4, Pregna-5,16-dien-20-one, 3β-hydroxy- 1769-67-1, Pregn-5-en-20-one, 3β-hydroxy-16β-methyl-, acetate 1808-63-5, Pregna-5,16-dien-20-one, 3β-hydroxy-16-methyl- 1863-41-8, Pregn-5-en-20-one, 3β-hydroxy-16α-methyl-, acetate 2193-00-2, 5α-Pregnan-20-one, 5,6α-epoxy-3β-hydroxy- 2857-83-2, 5α-Pregnan-20-one, 5,6α-epoxy-3β,17-dihydroxy-16α-methyl-, 3-acetate 5456-44-0, 5α-Pregnan-20-one, 3β,17-dihydroxy-, 3-acetate 13116-52-4, Pregn-5-en-20-one, 3β-hydroxy-16α,17-dimethyl-, acetate 13643-93-1, 5β-Pregnan-20-one, 16α,17-epoxy-3α,11β-dihydroxy-, cyclic ethylene acetal 14148-09-5, 5α-Pregnan-20-one, 5,6α-epoxy-3β-hydroxy-, acetate 14231-06-2, 5α-Pregnan-20-one, 5,6α:16α,17-diepoxy-3β-hydroxy-, acetate 14279-42-6, 5α-Pregn-16-en-20-one, 5,6α-epoxy-3β-hydroxy-, acetate 16394-71-1, Pregn-5-en-20-one, 3β-hydroxy-16α-methyl- 40148-10-5, Pregn-5-en-20-one, 3β-hydroxy-, cyclic ethylene acetal, acetate 55334-07-1, 5α-Pregnan-20-one, 3β,5-dihydroxy-6β,16α-dimethyl- 56630-86-5, 5α-Pregnan-20-one, 3β,5-dihydroxy-6β-methyl- 56784-22-6, 5α-Pregnan-20-one, 5,6α-epoxy-3β-hydroxy-, cyclic ethylene acetal, acetate 56784-24-8, 5α-Pregnan-20-one, 5,6α-epoxy-3β-hydroxy-16α-methyl-, cyclic ethylene acetal, acetate 56792-49-5, Pregn-5-en-20-one, 3β-hydroxy-16α-methyl-, cyclic ethylene acetal, acetate 101635-49-8, Pregn-5-en-20-one, 3β-hydroxy-6,15α-dimethyl-, acetate
(chromatography of)

The faster moving component was isolated by thick layer chromatography (silica gel H or alumina plates, 10% ethyl acetate-benzene) and had mp 140–142°. Attempts to isolate the slower moving component were unsuccessful, the band containing significant amounts of the faster moving substance.

When the isoflavanol (1.0 g), mp 130–139°, was oxidized by the Jones procedure, 7-benzyloxy-4'-methoxyisoflavanone (0.57 g), mp 130.5–132.5°, was obtained as needles after recrystallization from methanol. The infrared spectrum contained major bands at 1689 (s), 1620 (s), 1587 (s), and 1521 (s), cm^{-1}. The ultraviolet spectrum contained λ_{max}^{MeOH} 315 mμ (log ε 3.95) and 274 mμ (log ε 4.27).

Anal. Calcd for $C_{23}H_{20}O_4$ (360.4): C, 76.65; H, 5.59. Found: C, 76.61; H, 5.55.

C. **7-Benzyloxy-4'-methoxy-Δ³-isoflavene.**—A solution of the isoflavanol (4.0 g), mp 131–139°, in chloroform (200 ml) was stirred at 0–5° and a stream of dry hydrogen chloride passed through with the aid of a gas dispersion tube. The reaction was allowed to proceed for 4 hr, after which tlc (10% ethyl acetate-benzene) indicated complete dehydration. The deep orange organic solution was washed repeatedly with water until the aqueous layer afforded a pH reading of 5 and the organic layer was nearly colorless. After drying over a mixture of anhydrous sodium sulfate and sodium carbonate, the solvent was removed under reduced pressure to give a white, crystalline residue that, when dissolved in a minimum amount of boiling benzene and precipitated with methanol, provided a chromatographically uniform, white, microcrystalline solid (3.8 g), mp 142–150°. When recrystallized from benzene, the product was isolated as colorless plates (2.0 g), mp 154–155°. The infrared spectrum contained major bands at 1605 (s), 1570 (m), and 1500 (s) cm^{-1}. The ultraviolet spectrum contained λ_{max}^{MeOH} 335 mμ (log ε 4.43) and 251 mμ (log ε 4.22).

Anal. Calcd for $C_{23}H_{20}O_3$ (344.4): C, 80.21; H, 5.85. Found: C, 80.48; H, 6.02.

Hydrogenation of 7-Benzyloxy 4'-methoxy-Δ³-isoflavene.—A suspension of the Δ³-isoflavene (0.3 g, 0.874 mmole) in glacial acetic acid (20 ml) was hydrogenated at atmospheric pressure over 10% palladium-charcoal (50 mg). Complete dissolution of starting material was observed after approximately 43 ml of hydrogen had been consumed (theoretical value for 1 mole = 20.8 ml). The solution was filtered free of the catalyst by gravity and the filtrate was diluted with water to precipitate a crystalline solid. This solid consisted of two components (tlc, benzene-PMA), which were facilely separated by fractional crystallization. When dissolved in methanol (10 ml), 7-benzyloxy-4'-methoxyisoflavan (53 mg), mp 126–127.5°, crystallized as chromatographically pure platelets. The infrared spectrum contained bands at 1620 (s), 1585 (m), and 1510 (s) cm^{-1}. The ultraviolet spectrum contained λ_{max}^{MeOH} 284 mμ (log ε 3.76) and inflections at 289 (3.63) and 280 (3.73). Another crop (10 mg) was obtained by careful concentration of the mother liquor.

Anal. Calcd for $C_{23}H_{22}O_3$ (346.4): C, 79.74; H, 6.40. Found: C, 79.51; H, 6.74.

The mother liquor from the above recrystallization was allowed to evaporate to dryness at room temperature, and the white, crystalline residue was recrystallized from benzene-petroleum (bp 30–60°) ether to give 7-hydroxy-4'-methoxyisoflavan (66 mg), mp 157–158.5°, with slight softening at 153°. This crop was chromatographically uniform in two solvent systems, benzene and 10% ethyl acetate-benzene. The infrared spectrum contained major bands at 3424 (s), 1605 (s), 1590 (s), and 1500 (s) cm^{-1}. The ultraviolet spectrum contained λ_{max}^{MeOH} 284 mμ (log ε 3.73) and inflections at 289 (3.67) and 279 (3.69); $\lambda_{max}^{0.1\,N\,NaOH}$ 296 mμ (log ε 3.72) and inflections at 286 (3.70) and 278 (3.60). A second crop (17 mg) was isolated as a result of further dilution with petroleum ether. Tlc showed this fraction to be contaminated to a very slight extent by the benzyloxy derivative.

Anal. Calcd for $C_{16}H_{16}O_3$ (256.3): C, 74.98; H, 6.29. Found: C, 75.14; H, 6.31.

Registry No.—**2a**, 10499-07-7; **2b**, 10499-08-8; **2c**, 10499-09-9; **4a**, 10499-10-2; **4b**, 10499-11-3; **6**, 10499-12-4; **7a**, 10499-13-5; **7b**, 10499-14-6; 7-hydroxy-4'-methoxy-Δ³-isoflavene, 10535-63-4; 7-benzyloxy-4'-methoxyisoflavanol, 10535-64-5; 7-benzyloxy-4'-methoxyisoflavone, 10499-15-7; 7-benzyloxy-4'-methoxy-Δ³-isoflavene, 10499-16-8; 7-benzyloxy-4'-methoxyisoflavan, 10535-65-6; 7-hydroxy-4'-methoxyisoflavan, 10499-17-9.

The Synthesis of 17-Bromo-16α-methylprogesterones

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Received February 14, 1967

In situ bromination of 16α-methylpregnane-20-magnesium enolates gives a mixture of the 17α-bromo- and 17β-bromo-20-keto derivatives. Utilizing this reaction, 17α-bromo-16α-methylprogesterone and 17β-bromo-16α-methyl-17-isoprogesterone were prepared from 16-dehydropregnenolone acetate.

In recent years there has been considerable interest in the synthesis of 17α-bromoprogesterone[1] and some of its 6-substituted derivatives[2-5] in view of the enhanced progestational activity exhibited by this class of compounds. An earlier communication from these laboratories[6] described the preparation of a number of 16-alkylated progesterones. We now report the synthesis of 17α-bromo-16α-methylprogesterone (VI) and 17β-bromo-16α-methyl-17-isoprogesterone (VII).

The reaction of 16-dehydropregnenolone acetate (I) with methylmagnesium bromide in the presence of cuprous chloride, followed by *in situ* bromination of the resulting Grignard complex, afforded, after treatment with sodium iodide to debrominate any 5,6-dibromide, a mixture of 17α-bromo-16α-methylpregnenolone acetate (II)[7] and 17β-bromo-16α-methyl-17-isopregnenolone acetate (III). The 17α-bromo compound II, the major component of the mixture (90–95%), was obtained pure after column chromatography on silica gel. Fermentation of II with a culture of *Flavobacterium dehydrogenans*[6,8,9] gave 17α-bromo-16α-methylprogesterone (VI).

Direct dehydrohalogenation of the bromination mixture (II and III) with lithium bromide and lithium

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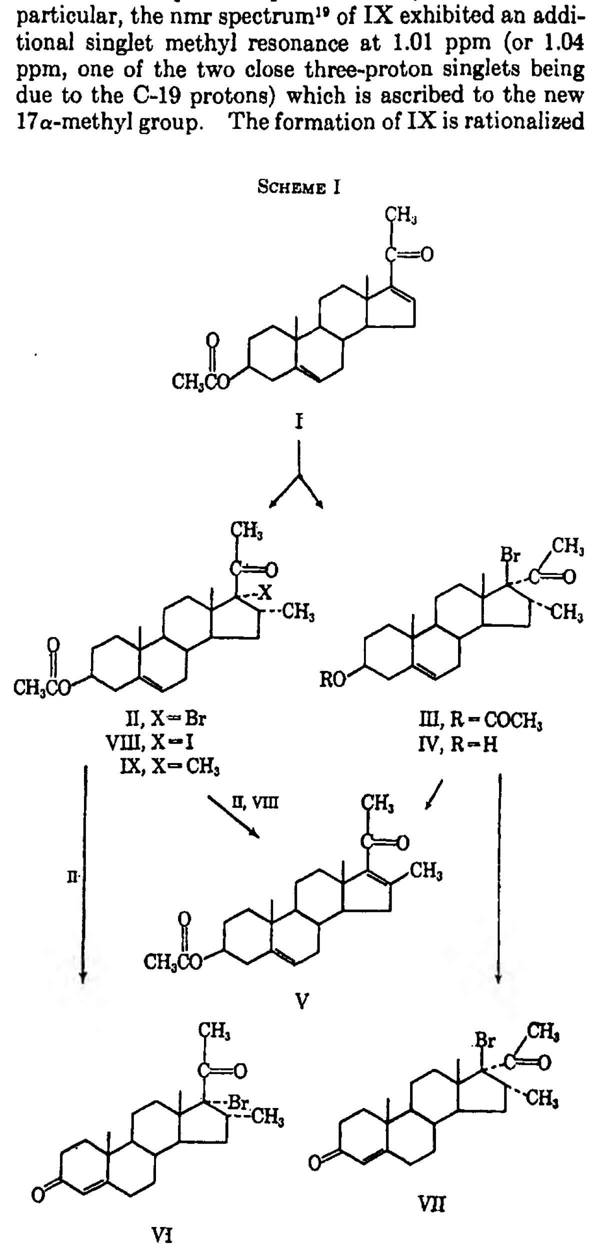
carbonate in dimethylformamide[7,10] resulted in selective reaction of II to give 16-methyl-16-dehydropregnenolone acetate (V).[11] The 17β-bromo isomer III was isolated from the dehydrobromination mixture by selective crystallization of V, followed by chromatography of the liquors on silica gel. The purity of compounds II and III was established by their nmr spectra, in which the 17β-bromo compound III exhibits a downfield shift of 19 cps for the C-18 methyl protons compared with the 17α-bromo isomer II (1.15 and 0.83 ppm, respectively).[12]

The structure of III follows from the method of synthesis and from the dehydrobromination under more forcing conditions in refluxing dimethylacetamide, to give the 16-methyl-16-dehydro compound V. Rotational differences between II and III (see the Experimental Section) and the infrared spectra are in accord with this assignment. It is of interest that 17β-bromo derivatives have not previously been reported from the bromination of 20-keto steroids or their enol derivatives. In fact, a study by Wendler and coworkers[13] showed that in the 16-desmethyl series such brominations give the pure 17α-bromo analogs, while Heusler and co-workers[14] found no evidence of 17,20β-oxide formation in the epoxidation of a 16α-methyl-17(20)-enol acetate. On the other hand, 17β attack has been observed to a minor extent in the reaction of 17-keto steroids with potassium acetylide,[15] sodium phenylacetylide,[16] and methyl Grignard reagent.[17] The reaction of 5α-9(11),16-pregnadien-3β-ol-20-one acetate with methyl Grignard reagent followed by *in situ* bromination as described above similarly gave a mixture of the 17α-bromo-16α-methyl derivative and 5 to 10% of the 17β-bromo isomer.[18]

Hydrolysis of compound III with potassium hydroxide gave the 3β-hydroxy analog IV, which on Oppenauer oxidation was converted to 17β-bromo-16α-methyl-17-isoprogesterone (VII). Alternatively, III was fermented with a culture of *Flavobacterium dehydrogenans* to afford VII directly, albeit in rather low yield. (See Scheme I.)

The synthesis of 17-iodo-20-keto steroids has not been reported. In connection with the present work it was desirable to prepare a 17-iodoprogesterone derivative. The addition of iodine to the Grignard complex from I, *in situ*, afforded 17α-iodo-16α-methylpregnenolone acetate (VIII), the stereochemistry at C-17 being assigned on the basis of rotation and predominant α attack. Dehydroiodination of VIII with lithium bromide and lithium carbonate in dimethylformamide yielded the 16-dehydro compound V. In addition to VIII, there was isolated from the iodination a minor, halogen-free product which was assigned structure IX, 16α,17α-dimethyl-5-pregnen-3β-ol-20-one 3-acetate, on the basis of spectroscopic and analytical data. In particular, the nmr spectrum[19] of IX exhibited an additional singlet methyl resonance at 1.01 ppm (or 1.04 ppm, one of the two close three-proton singlets being due to the C-19 protons) which is ascribed to the new 17α-methyl group. The formation of IX is rationalized

SCHEME I



as proceeding by methylation of the intermediate Grignard enolate complex[20] by methyl iodide, the latter being formed on reaction of excess methyl Grignard reagent with iodine. Compound VIII was rather unstable and attempts to convert it to a pure progesterone derivative, either chemically or microbiologically, were unsuccessful.

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(12) These nmr spectra were determined on a Varian A-60 instrument in deuteriochloroform. We express our thanks to Professor L. Mandell for obtaining the nmr spectra.

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(19) This spectrum was determined on a Varian A-60A instrument in deuteriochloroform. We thank Mr. M. Yudis of the Physical Organic Chemistry Department, Schering Corp., for the measurement and for helpful discussions.

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Experimental Section[21]

17α-Bromo-16α-methyl-5-pregnen-3β-ol-20-one Acetate (II).—To a solution of methylmagnesium bromide, prepared from 0.240 g of magnesium turnings and methyl bromide, in 20 ml of tetrahydrofuran, was added under argon, with stirring, 50 mg of cuprous chloride, followed by a solution of 1.75 g of 16-dehydropregnenolone acetate in 10 ml of tetrahydrofuran. The mixture was stirred at room temperature for 30 min, then cooled in an ice bath, and a solution of 2.37 g of bromine in 5 ml of carbon tetrachloride was added with stirring at a rapid dropwise rate. The mixture was stirred for 5 min, then 50 ml of ether was added, and the excess bromine was reduced by the addition of 10% aqueous sodium bisulfite solution. The organic phase was separated, washed with 5% sodium bicarbonate solution followed by saturated sodium chloride solution, dried, and concentrated to a residue under reduced pressure. The residue was dissolved in 50 ml of acetone, 3.0 g of sodium iodide was added, and the mixture kept at room temperature for 16 hr. It was then decolorized with 10% aqueous sodium bisulfite solution and poured into cold water. The crystalline precipitate was filtered, washed with water, and dried to give 2.19 g of crude II, containing 5–10% of the 17β-bromo isomer III. A sample of 1.00 g of the crude product was chromatographed on silica gel. Fractions eluted with 2% ether–hexane were combined and crystallized from methylene chloride–methanol to give II (0.867 g), mp 185–187°. The analytical sample was recrystallized from the same solvent system: 185°; $[\alpha]_D$ −126°; $\lambda\lambda_{max}^{Nujol}$ 5.78, 5.87, 8.10 μ (lit.[7] mp 174–176°).

Anal. Calcd for $C_{24}H_{35}BrO_3$: C, 63.85; H, 7.82; Br, 17.70. Found: C, 63.78; H, 8.01; Br, 17.48.

17β-Bromo-16α-methyl-17-iso-5-pregnen-3β-ol-20-one Acetate (III).—To a stirred suspension of 69.9 g of anhydrous lithium bromide and 60.0 g of anhydrous lithium carbonate in 1100 ml of dry dimethylformamide was added 65.0 g of the crude mixture of II and III (prepared as above) and the mixture was heated under reflux with stirring for 1 hr. It was then cooled and poured into cold, dilute hydrochloric acid and the resulting precipitate was filtered, washed well with water, and dried to give 53.0 g of crude material. The latter was taken up in ether–methylene chloride, treated with decolorizing carbon, and crystallized with methanol, affording 29.7 g of 16-methyl-5,16-pregnadien-3β-ol-20-one acetate (V), mp 171–172°. A sample was recrystallized from methylene chloride–methanol: mp 173–174°; λ_{max}^{MeOH} 252 mμ (ε 9000); infrared spectrum identical with that of an authentic sample (lit.[11] mp 176–178°).

The filtrate from the original crystallization was concentrated to a residue which was chromatographed on silica gel. Fractions eluted with 2% ether–hexane were combined and crystallized from ether–methanol to give a total of 3.02 g of the 17β-bromo compound III, mp 142–143°. The analytical sample was recrystallized from ether–methanol: mp 143°; $[\alpha]_D$ +10°; $\lambda\lambda_{max}^{Nujol}$ 5.73, 5.87, 8.00 μ.

Anal. Calcd for $C_{24}H_{35}BrO_3$: C, 63.85; H, 7.82; Br, 17.70. Found: C, 63.55; H, 7.63; Br, 17.53.

Dehydrobromination of 17β-Bromo-16α-methyl-17-iso-5-pregnen-3β-ol-20-one Acetate (III).—To a solution of 100 mg of III in 5 ml of dry N,N-dimethylacetamide was added 235 mg of anhydrous lithium bromide and 190 mg of anhydrous lithium carbonate. The mixture was heated under reflux for 3.5 hr, then poured into 10% hydrochloric acid–ice, and the resulting precipitate was filtered, washed well with water, and dried, giving 75 mg of crude product. Crystallization from ether–methanol, after treatment with decolorizing charcoal, afforded 36 mg of 16-methyl-5,16-pregnadien-3β-ol-20-one acetate (V): mp 172–173°; infrared spectrum identical with that of an authentic sample.

17α-Bromo-16α-methyl-4-pregnene-3,20-dione (17α-Bromo-16α-methylprogesterone) (VI).—17α-Bromo-16α-methyl-5-pregnen-3β-ol-20-one acetate (500 mg) was incubated with a culture of *Flavobacterium dehydrogenans* for 67 hr. The fermentation mixture was extracted with chloroform and the extracts were concentrated to a residue which was crystallized from acetone, after treatment with decolorizing charcoal, to give 231 mg, mp 192–195° dec. Crystallization from methylene chloride–methanol afforded VI (199 mg), mp 199–203° dec. The analytical sample was further purified by thick layer chromatography on silica gel. The ultraviolet absorbing zone was eluted with methylene chloride and the product was crystallized with methanol: mp 197–201° dec; $[\alpha]_D$ −36°; λ_{max}^{MeOH} 240 mμ (ε 16,900); $\lambda\lambda_{max}^{Nujol}$ 5.86, 6.01, 6.16 μ.

Anal. Calcd for $C_{22}H_{31}BrO_2$: C, 64.86; H, 7.67; Br, 19.62. Found: C, 64.76; H, 7.64; Br, 20.00.

17β-Bromo-16α-methyl-17-iso-5-pregnen-3β-ol-20-one (IV).—A solution of 500 mg of compound III in a mixture of 30 ml of a 5% solution of potassium hydroxide in 95% aqueous methanol and 5 ml of methylene chloride was stirred under nitrogen at room temperature for 1 hr. The mixture was diluted with ice water, acidified with acetic acid, and extracted with methylene chloride. The extracts were concentrated and the product was crystallized from acetone–hexane to afford IV (410 mg), mp 197°. Recrystallization from acetone–hexane gave the analytical sample: mp 197–198°; $[\alpha]_D$ +10°; $\lambda\lambda_{max}^{Nujol}$ 2.82, 5.90 μ.

Anal. Calcd for $C_{22}H_{33}BrO_2$: C, 64.54; H, 8.12; Br, 19.52. Found: C, 64.80; H, 8.30; Br, 19.58.

17β-Bromo-16α-methyl-17-iso-4-pregnene-3,20-dione (17β-Bromo-16α-methyl-17-isoprogesterone) (VII). A. From IV by Oppenauer oxidation.—To a solution of 600 mg of the 3β-hydroxy-Δ^5-compound IV in 12 ml of anhydrous toluene containing 2.5 ml of cyclohexanone was added a solution of 500 mg of aluminum isopropoxide in 10 ml of toluene and the stirred mixture was heated under reflux for 1.5 hr. The mixture was slightly cooled and 2 ml of water was added. The excess water was azeotroped off, the cooled solution was filtered, and the filtrate was steam distilled. The solid obtained on cooling of the pot residue was filtered and chromatographed on silica gel. Fractions eluted with 8% ether–hexane were combined and crystallized from methylene chloride–methanol to give a total of 390 mg of VII, mp 172–173°. Recrystallization afforded the analytical sample: mp 173°, $[\alpha]_D$ +133°; λ_{max}^{MeOH} 239 mμ (ε 17,600); $\lambda\lambda_{max}^{Nujol}$ 5.90, 5.99, 6.21 μ.

Anal. Calcd for $C_{22}H_{31}BrO_2$: C, 64.86; H, 7.67; Br, 19.62. Found: C, 64.65; H, 7.79; Br, 19.36.

B. From III by Fermentation with *Flavobacterium dehydrogenans*.—A sample of 500 mg of the 3-acetate III was incubated with a culture of *Flavobacterium dehydrogenans* for 12 hr. The fermentation mixture was extracted with chloroform and the extracts were concentrated to a residue which was taken up in acetone, treated with decolorizing charcoal, and crystallized from acetone–methanol to afford a total of 273 mg of crude VII. The material was chromatographed on silica gel, giving 128 mg of pure VII: mp 170–172°; $[\alpha]_D$ +133°; λ_{max}^{MeOH} 241 mμ (ε 16,900); infrared spectrum very similar to that of VII prepared as described in part A.

17α-Iodo-16α-methyl-5-pregnen-3β-ol-20-one Acetate (VIII) and 16α,17α-Dimethyl-5-pregnen-3β-ol-20-one Acetate (IX).—To a solution of methylmagnesium bromide, prepared from 0.240 g of magnesium in 20 ml of tetrahydrofuran, containing 50 mg of cuprous chloride, was added with stirring a solution of 1.75 g of 16-dehydropregnenolone acetate in 10 ml of tetrahydrofuran. The mixture was stirred at 10° for 25 min; then a solution of 2.5 g of iodine in 10 ml of tetrahydrofuran was added dropwise until a permanent coloration was obtained (about 5 ml of the solution was added). The mixture was stirred for 5 min, then poured into cold water. The resulting precipitate was filtered, suspended in ether, and shaken with a saturated, aqueous solution of ammonium chloride. The latter was separated and washed with ether and the ethereal solutions were combined, washed with ammonium chloride solution and water, dried, and concentrated to an oil which solidified on cooling. Trituration with cold methanol gave 1.90 g of solids. Chromatography of 600 mg of the crude product on silica gel and elution with 2% ether–hexane, followed by crystallization from methanol gave 400 mg of VIII: mp 129° dec; $[\alpha]_D$ −148°; $\lambda\lambda_{max}^{Nujol}$ 5.76, 5.90, 8.05 μ.

Anal. Calcd for $C_{24}H_{35}IO_3$: C, 57.83; H, 7.08; I, 25.46. Found: C, 57.87; H, 7.20; I, 25.02.

Further elution with the same solvent system gave, after a short break, additional solid fractions which were combined and crystallized from methylene chloride–methanol to afford 45 mg of IX: mp 218–219°; $[\alpha]_D$ −64°; $\lambda\lambda_{max}^{Nujol}$ 5.76, 5.90, 8.12 μ.

Anal. Calcd for $C_{25}H_{38}O_3$: C, 77.67; H, 9.91. Found: C, 77.36; H, 9.79.

Dehydroiodination of 17α-Iodo-16α-methyl-5-pregnen-3β-ol-20-one Acetate (VIII).—To a solution of 200 mg of 17α-iodo compound VIII in 5 ml of anhydrous dimethylformamide was added 235

(21) Melting points were determined on a Fisher-Johns apparatus. Rotations were measured in dioxane. The physical and analytical data were obtained by the Physical Chemistry Department, Schering Corp. Purity of compounds was checked by thin layer chromatography on silica gel.

mg of dry lithium bromide and 190 mg of dry lithium carbonate. The mixture was heated under reflux for 45 min, then cooled, and poured into dilute hydrochloric acid. The precipitate was filtered, washed, and dried, giving 124 mg of crude V. Chromatography on silica gel, elution with 2% ether-hexane, and crystallization from methylene chloride-methanol gave 38 mg of 16-dehydro-16-methylpregnenolone acetate (V): mp 170-172°; λ_{max}^{MeOH} 251 mμ (ε 8600); infrared spectrum matches that of an authentic sample.

Registry No.—II, 983-23-3; III, 13116-47-7; IV, 13116-48-8; V, 982-06-9; VI, 13116-50-2; VII, 13116-51-3; VIII, 13143-64-1; IX, 13116-52-4.

Acknowledgments.—We thank Dr. E. B. Hershberg for helpful suggestions in the course of this work. We are indebted to Miss C. Federbush for carrying out the microbiological transformations.

Chemistry of Erythronolide B. Acid-Catalyzed Transformations of the Aglycone of Erythromycin B

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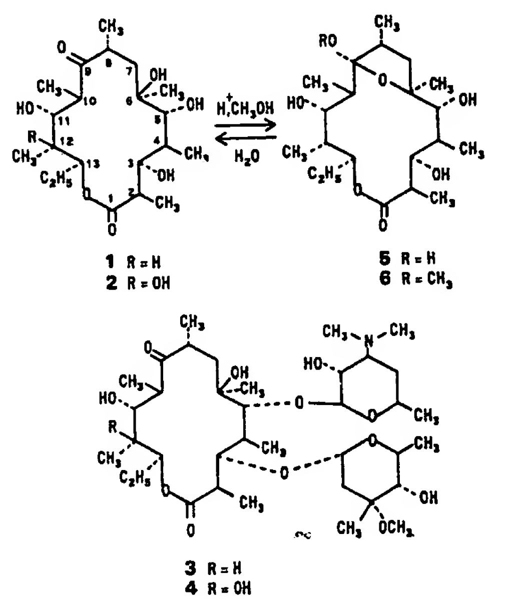
Received January 17, 1967

The treatment of erythronolide B and its derivatives with aqueous acid establishes an equilibrium between the hydroxy ketone and its corresponding 6,9-hemiketal or derived enol ether. An enol ether has been isolated from the treatment of triacetylerythronolide B. Further reaction of this enol ether with anhydrous acid caused elimination of the allylic acetoxy group giving a conjugated enol ether. Hydrolysis of this compound led to the formation of two anhydroerythronolide B derivatives, one of which was a member of the previously unknown 8-*epi*-erythronolide B series.

Erythronolide B (1)[1] is the 14-membered lactone portion of erythromycin B[4] (3), one of the macrolide family of antibiotics.[5] It has been shown to be an effective biological precursor of the erythromycins.[6] Even though erythromycin B has been shown to be more stable to acid than erythromycin A,[7] it is not possible to obtain erythronolide B by removal of the sugars, cladinose and desosamine, because the lactone ring is degraded during the severe hydrolysis procedure needed to remove the amino sugar. Similarly, erythronolide A (2) cannot be obtained from erythromycin A (4) because of extensive acid-catalyzed degradation.[8] Since we had available to us a large supply of erythronolide B from a fermentation procedure,[9] we decided to examine the nature of the acid-catalyzed degradation of this ring system (Scheme I).

SCHEME I



It has been postulated that the acid-catalyzed degradation of erythromycin A involves the formation of a hemiketal bond between the carbonyl at C-9 and one of the tertiary hydroxyls at C-6 or C-12, followed by or concomitant with participation of the second tertiary hydroxyl giving a spiroketal.[8] Erythromycin B (and erythronolide B) does not have the tertiary hydroxyl at C-12, precluding the possibility of spiro ketal formation.[4] It should be possible, however, for a hemiketal bond to form between the carbonyl and the tertiary hydroxyl at C-6, and formation of the hemiketal 5 may be the first step in the acid-catalyzed degradation of the erythronolide B ring system. Since the ultraviolet absorption spectrum of erythronolide B contains an absorption maximum at 288 mμ (ε 39) due to the C-9 ketone, it was felt that the formation of a hemiketal could be followed by observing the disappearance of this peak.[10] Solutions were made of erythronolide B (1%) in methanol containing different concentrations of hydrochloric acid, and the absorbance of each solution at 288 mμ was recorded as a function of time. The rate of decrease in absorbance was very fast, even at low concentrations of acid. The half-life of the ketone function at room temperature in 10^{-4} *M* methanolic hydrochloric acid was slightly less than 4 min.

(1) Stereochemistry is based on that determined for erythromycin A.[2,3] The structural formula used does not imply a particular conformation of the molecule but is merely a convenient planar representation of the 14-membered ring. Similarly, the double-bond geometry of the olefinic derivatives is not specified by the use of this planar structure.
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(9) P. L. Tardrew and M. A. Nyman, U. S. Patent 3,127,315 (1964).
(10) This experiment was suggested by Dr. P. H. Jones. Preliminary observations of this effect were carried out by Dr. M. A. Nyman.

Compound (VI)

CAS Registry Record:

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RN     5297-33-6  REGISTRY
ED     Entered STN:  16 Nov 1984
CN     Pregn-5-en-20-one, 3-(acetyloxy)-16-ethyl-, (3β,16α)- (9CI)
       (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN     Pregn-5-en-20-one, 16α-ethyl-3β-hydroxy-, acetate (6CI, 7CI,
       8CI)
FS     STEREOSEARCH
MF     C25 H38 O3
LC     STN Files:   BEILSTEIN*, CA, CAOLD, CAPLUS, CHEMCATS, USPATFULL
           (*File contains numerically searchable property data)
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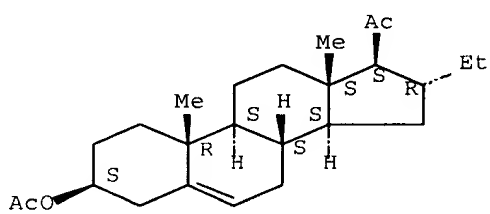
Absolute stereochemistry.

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                    6 REFERENCES IN FILE CA (1907 TO DATE)
                    6 REFERENCES IN FILE CAPLUS (1907 TO DATE)
                    2 REFERENCES IN FILE CAOLD (PRIOR TO 1967)
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Oldest Refs in Chem. Abs.:

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L21    ANSWER 1 OF 2  CAOLD  COPYRIGHT 2006 ACS on STN
AN     CA64:6715d  CAOLD
TI     Beckmann rearrangement of pregna-5-16-dien-3β-ol-20-one acetate with
       BF3
AU     Romo, Jesus; Romo de Vivar, A.
IT      853-23-6    5040-79-9    5040-81-3    5040-82-4    5040-83-5    5040-84-6
       5040-85-7    5040-86-8    5040-87-9    5040-88-0    5040-89-1    5040-90-4
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       5297-32-5    5297-33-6    5297-34-7    5488-41-5    6083-95-0
       6083-96-1   96275-48-8
```

III, but in a similar expt. the isome 14β,17α-4-pregnene-3,13,-15,20-tetraone, m. 218.5–21° (aq. e₂CO), $[\alpha]_D^{26}$ 155° ± 2° ($CHCl_3$), was obtained; it was also formed when 21 mg. III was treated in 1 ml. MeOH with 25 mg. p-$MeC_6H_4SO_3H$ 3.5 hrs. at 22°. The N.M.R. data of these compds. in $CDCl_3$ are given and are discussed. F. E. Brauns

The Beckmann rearrangement of pregna-5,16-dien-3β-ol-20-one acetate with boron trifluoride. J. Romo and A. Romo de Vivar (Univ. Anatomia, Mexico, D.F.). *Rev. Soc. Quim. Mex.* 6(3), 77–86(1962)(Span). The Beckmann rearrangement of 20-acetoximinopregna-5,16-dien-3β-ol acetate catalyzed with BF_3 in benzene as solvent gave dehydroepiandrosterone as final product. However, when the reaction was performed using as solvent Ac_2O, 17β-methyl-18-norisopregna-5,13-diene-3β,16α-diol-20-one diacetate (I) and 16-acetyl-17-acetamidoandrosta-5,16-dien-3β-ol (II) were obtained. I had λ 5.80 μ with inflection in 5.88 μ. I formed an oxime and gave a diepoxide when treated with perbenzoic acid. II was shown to contain N. For further identification several derivs. of both I and II were prepd. and identified. The reaction is useful to prep. androstane derivs. starting from Δ^{16}-20-ketones. R. Mancilla

Synthesis of 17α-hydroxyprogesterone caproate. A. M. Veitsman and N. T. Moleva (Med. Inst., Lvov). *Farmatsevt. Zh.* (Kiev) 20(1), 10–13(1965)(Ukrain). 17α-Hydroxyprogesterone

L21 ANSWER 2 OF 2 CAOLD COPYRIGHT 2006 ACS on STN
AN CA55:1697a CAOLD
TI Beckmann rearrangement of the acetoxime of 5,16-pregnadien-3β-ol-20-one acetate with BF3
AU Romo, Jesus; Romo de Vivar, A.
IT 853-23-6 5040-79-9 5040-81-3 5040-84-6 5040-85-7 5040-86-8
5040-87-9 5040-88-0 5040-90-4 5040-91-5 5040-92-6 5040-93-7
5040-94-8 5040-95-9 5088-59-5 5088-60-8 5088-61-9 5088-62-0
5088-63-1 5154-40-5 5297-31-4 5297-32-5 5297-33-6
5297-34-7 5488-41-5 118726-04-8

1961 1697 *10J—Sterc*

3060–2800 cm.$^{-1}$ where V showed only 3 bands. C. R. Addinall

Beckmann rearrangement of the acetoxime of 5,16-pregnadien-3β-ol-20-one acetate with boron trifluoride. J. Romo and A. Romo de Vivar (Univ. Mexico, Mexico City). *J. Am. Chem. Soc.* 81, 3446–52(1959).—Beckmann rearrangement of 3β-acetoxy-5,16-pregnadien-20-one acetoxime with BF_3–Et_2O in benzene yielded dehydroepiandrosterone acetate, whereas in Ac_2O the rearrangement followed a different course and two products were isolated: 17β-methyl-18-nor-5,13(14)-isopregnadiene-3β,16α-diol-20-one diacetate (I) and 16-acetyl-17-acetamido-5,16-androstadien-3β-ol acetate (II). Several derivs. of these compds. were prepd. in the process of establishing their structure. $LiAlH_4$ redn. of I afforded the 3,16,20-triol, m. 202–204°, [α] −207°. The oxo group in I was eliminated by hydrogenolysis of the cycloethylene mercaptol, m. 137–40° $[\alpha]_D^{20}$ −108°, whereupon the diacetate, m. 123–4°, $[\alpha]_D^{20}$ −176°, was obtained. $KHCO_3$ sapon. of the diacetate gave the monoacetate which, on Oppenauer oxidn., afforded the Δ^4-3-ketone. II was hydrolyzed with KOH to remove the acetate groups and the latter benzoylated with BzCl in pyridine to give the dibenzoate, m. 279–81°, $[\alpha]_D^{20}$ −44°. Benzoylation by the Schotten-Baumann method gave the monobenzoate (III), m. 218–20°, $[\alpha]_D^{20}$ −51°. Oppenauer oxidn. of III yielded 16-acetyl-17-benzamido-4,16-androstadien-3-one, m. 221–2°, $[\alpha]_D^{20}$ 94°. D. P. Langlois

Compounds related to the steroid hormones. I. Preparation and reactions of some 11-methyl steroids. J. Elks (Glaxo Labs. Ltd., Greenford, Engl.) J. Chem.

L24 ANSWER 1 OF 1 HCA COPYRIGHT 2006 ACS on STN

AN 119:117611 HCA Full-text
TI Methylation or ethylation agents comprising trimethylaluminum, tiethylaluminum, or diethyl zinc and catalytic copper compounds for conjugate addition reactions
IN Westermann, Juergen; Nickisch, Klaus
PA Schering A.-G., Germany
SO Eur. Pat. Appl., 18 pp.
CODEN: EPXXDW
DT Patent
LA German
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE	
PI	**EP 534582**	A1	19930331	EP 1992-250276	19920928	<--
	EP 534582	B1	19970108			
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE					
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	CA 2120004	C	20060110			
	WO 9306066	A1	19930401	WO 1992-EP2227	19920928	<--
	W: CA, JP, US					
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	US 5908944	A	19990601	US 1994-211230	19940930	<--
PRAI	DE 1991-4132755	A	19910927			
	WO 1992-EP2227	W	19920928			

OS CASREACT 119:117611; MARPAT 119:117611
AB Ethylating or methylating agents consist of Me3Al, Me2Zn or Et3Al combined with catalytic quantities of ≥1 Cu(I) and/or Cu(II) compound Thus, a mixture of androsta-1,4-dien-3,17-dione and CuBr in dioxane was treated with Me3Al in PhMe at ≤ 35° to give 77% 1α-methylandrost-4- en-3,17-dione.

L25 ANSWER 3 OF 4 HCA COPYRIGHT 2006 ACS on STN
AN 64:36099 HCA Full-text
OREF 64:6715d-f
TI The Beckmann rearrangement of pregna-5,16-dien-3β-ol-20-one acetate with boron trifluoride
AU Romo, J.; de Vivar, A. Romo
CS Univ. Anatomia, Mexico, D.F.
SO Revista de la Sociedad Quimica de Mexico (1962), 6(3), 77-86
CODEN: RSQMAN; ISSN: 0583-7693
DT Journal
LA Spanish
AB The Beckmann rearrangement of 20-acetoximinopregna-5,16-dien-3β-ol acetate catalyzed with BF3 in benzene as solvent gave dehydroepiandrosterone as final product. However, when the reaction was performed using as solvent Ac2O, 17β-methyl-18-norisopregna-5,13- diene-3β,16α-diol-20-one diacetate (I) and 16-acetyl-17-acetamidoandrosta-5,16-dien-3β-ol (II) were obtained. I had λ 5.80 μ with inflection in 5.88 μ. I formed an oxime and gave a diepoxide when treated with perbenzoic acid. II was shown to contain N. For further identification several derivs. of both I and II were prepared and identified. The reaction is useful to prepare androstane derivs. starting from Δ16-20-ketones.

L25 ANSWER 4 OF 4 HCA COPYRIGHT 2006 ACS on STN

AN 55:8299 HCA

Full Text

OREF 55:1697a-c

TI Beckmann rearrangement of the acetoxime of 5,16-pregnadien-3β-ol-20-one acetate with boron trifluoride

AU Romo, J.; de Vivar, A. Romo

CS Univ. Mexico, Mexico City

SO Journal of the American Chemical Society (1959), 81, 3446-52

CODEN: JACSAT; ISSN: 0002-7863

DT Journal

LA Unavailable

OS CASREACT 55:8299

AB Beckmann rearrangement of 3β-acetoxy-5,16-pregnadien-20-one acetoxime with BF3-Et2O in benzene yielded dehydroepiandrosterone acetate, whereas in Ac2O the rearrangement followed a different course and two products were isolated: 17β-methyl-18-nor-5,13(14)-isopregnadiene-3β, 16α-diol-20-one diacetate (I) and 16-acetyl-17-acetamido-5,16- androstadien-3β-ol acetate (II). Several derivs. of these compds. were prepared in the process of establishing their structure. LiAlH4 reduction of I afforded the 3,16,20-triol, m. 202-204°, [α] -207°. The oxo group in I was eliminated by hydrogenolysis of the cycloethylene mercaptol, m. 137-40°, [α]20D -108°, whereupon the diacetate, m. 123-4°, [α]20D -176°, was obtained. KHCO3 saponification of the diacetate gave the monoacetate which, on Oppenauer oxidation, afforded the Δ4-3-ketone. II was hydrolyzed with KOH to remove the acetate groups and the latter benzoylated with BzCl in pyridine to give the dibenzoate, m. 279-81°, [α]20D -44°. Benzoylation by the Schotten-Baumann method gave the monobenzoate (III), m. 218-20°, [α]20D -51°. Oppenauer oxidation of III yielded 16-acetyl-17-benzamido-4,16-androstadien-3- one, m. 221-2°, [α]20D 94°.

tered off and washed well with chloroform and the combined washings and filtrates were evaporated to dryness with the rotary evaporator. The brown solid residue was extracted with 100 ml. of boiling benzene, the benzene evaporated and the residue recrystallized from methanol to give 0.8 g. (50%) of pale yellow needles of 21-methoxy-4-formyltropone of m.p. 183–184°. The reported m.p. was 182–183°.

2-Methoxy-4-carboxytropone (XI).—To a cold basic suspension of fresh silver oxide, prepared by adding 6.8 g. (0.04 mole) of silver nitrate to a solution of 2.8 g. (0.07 mole) of sodium hydroxide dissolved in 100 ml. of water, was added 3.3 g. (0.02 mole) of 2-methoxy-4-formyltropone over 15 min. with stirring. The mixture was allowed to stand at ice-bath temperature for an additional half-hour then warmed to room temperature over one-half hour. The silver was filtered off and washed with water and the solution acidified with cold 10% hydrochloric acid. The precipitated acid was filtered off and recrystallized from 25% methanol in water to give 2.5 g. (70%) of pale yellow needles of 2-methoxy-4-carboxytropone, m.p. 253–254°. The reported m.p.[1] was 258°.

2-Amino-4-carboxytropone (XII).—2-Methoxy-4-carboxytropone (1.7 g.) and liquid ammonia (20 ml.) were sealed in a glass tube and the tube was stored in a steel bomb for 3 days at room temperature. (On some of the runs the tube exploded inside of the bomb. This could be largely prevented by charging the bomb with about 150 p.s.i. of N_2 after introducing the frozen (Dry Ice) tube and later cooling the entire apparatus to Dry Ice temperature before opening the bomb.) The contents of the tube were then transferred to a beaker and the ammonia was allowed to evaporate (caution: splattering). The solid residue was taken up in 25 ml. of water and acidified with 10% hydrochloric acid. The precipitated acid was filtered off and weighed 1.45 g. (93%). Three recrystallizations from aqueous ethanol gave the analytical sample as small orange needles, m.p. 280° d. (sealed tube).

Anal. Calcd. for $C_8H_7O_3N$: C, 58.18; H, 4.28. Found: C, 57.93; H, 4.41.

2-Acetamino-4-carboxytropone (XIII).—Treatment of 2-amino-4-carboxytropone with excess acetic anhydride in pyridine on the steam-bath for 45 min. followed by acidification with 10% hydrochloric acid in ice gave a yellow-brown solid. Two recrystallizations from ethanol with decolorizing charcoal gave a pale yellow powder of m.p. 230° dec. (sealed tube).

Anal. Calcd. for $C_{10}H_9O_4N$: C, 57.97; H, 4.38. Found: C, 57.50; H, 4.50.

Acid Chloride of 2-Acetamino-4-carboxytropone.—To a stirred suspension of 0.25 g. of the dry potassium salt of 2-acetamino-4-carboxytropone in 10 ml. of benzene was added 0.1 ml. of oxalyl chloride in 5 ml. of benzene. Stirring was continued for 3 hr. at room temperature during which time a gas was evolved and the solution became yellow. The mixture was filtered and evaporated to dryness on the rotary evaporator to give the crude acid chloride as a yellow solid. Treatment with *p*-bromoaniline in benzene gave the *p*-bromoanilide derivative which melted, after two recrystallizations from benzene, at 225–227°.

Anal. Calcd. for $C_{16}H_{13}O_3N_2Br$: C, 53.20; H, 3.63; Found: C, 53.52; H, 3.89.

2-Amino-6-styryltropone (VI).—A mixture of 1.5 g. of 2-methoxy-6-styryltropone (Va) and 30 ml. of liquid ammonia was allowed to stand in a sealed tube at room temperature for 45 hr. After evaporation of the ammonia the product was recrystallized from 100 ml. of benzene to give 0.85 g. of orange plates, m.p. 135–136°. An additional 0.35 g. was obtained by treating the filtrate with decolorizing charcoal and evaporating to 20 ml. The total yield was 1.2 g. (86%). Three more crystallizations from benzene (with charcoal treatment) gave the analytical sample as sparkling golden plates, m.p. 137–138°.

Anal. Calcd. for $C_{15}H_{13}NO$: C, 80.69; H, 5.87. Found: C, 80.24; H, 5.80.

The acetyl derivative VIa was prepared with acetic anhydride and pyridine, and melted, after three crystallizations from heptane containing 5% of benzene, at 179.5–181°.

Anal. Calcd. for $C_{17}H_{15}NO_2$: C, 76.96; H, 5.70. Found: C, 76.96; H, 5.98.

2-Hydrazino-6-styryltropone (VII).—To a suspension of 0.238 g. of 2-methoxy-6-styryltropone in 1 ml. of methanol and 1 ml. of dioxane was added 0.1 g. of 95% hydrazine. The mixture was warmed on the steam-bath for 5 min. and cooled; the crystals were collected (0.21 g., 88%) and after three crystallizations from methanol gave brilliant red crystals, m.p. 162.5–163.5°.

Anal. Calcd. for $C_{15}H_{14}N_2O$: C, 75.60; H, 5.92. Found: C, 75.91; H, 5.79.

4-(β-Carboxyvinyl)-tropolone (XIV).—A mixture of 0.75 g. of 4-formyltropolone, 0.52 g. of malonic acid, 1 ml. of piperidine and 20 ml. of pyridine was heated on the steam-bath for 12 hr. The resulting solution was poured over ice and acidified with 10% hydrochloric acid and the acidic solution was continuously extracted with ether for 24 hr. Evaporation of the dried ether solution and recrystallization of the solid residue from ethanol gave yellow needles of 4-(β-carboxyvinyl)-tropolone, m.p. 226.5–227.5° dec. (sealed tube). The compound gave an immediate green-brown color with ethanolic ferric chloride solution.

Anal. Calcd. for $C_{10}H_8O_4$: C, 62.60; H, 4.20. Found: C, 62.84; H, 4.37.

ROCHESTER 20, N. Y.

[CONTRIBUTION NO. 111 FROM THE INSTITUTO DE QUÍMICA DE LA UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO]

The Beckmann Rearrangement of the Acetoxime of $\Delta^{5,16}$-Pregnadien-3β-ol-20-one Acetate with Boron Trifluoride

BY J. ROMO AND A. ROMO DE VIVAR

RECEIVED DECEMBER 11, 1958

Beckmann rearrangement of $\Delta^{5,16}$-pregnadien-3β-acetoxy-20-acetoximino (Ib) with boron trifluoride etherate in benzene afforded dehydro epiandrosterone acetate (IIb), whereas in acetic anhydride the rearrangement followed a different course and two products were isolated: 17β-methyl-18-nor-$\Delta^{5,13(14)}$-isopregnadien-3β,16α-diol-20-one diacetate (IIIa) and 16-acetyl-17-acetylamino-$\Delta^{5,16}$-androstadien-3β-ol acetate (VIIa); several derivatives are described.

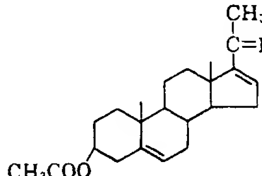
The Beckmann rearrangement of $\Delta^{5,16}$-pregnadien-3β-ol-20-one 3-acetate 20-oxime (Ia) has been studied by G. Rosenkranz, *et al.*[1] Using *p*-acetamidobenzenesulfonyl chloride as catalyst, the reaction afforded dehydroepiandrosterone (IIa) and working under appropriate conditions the intermediate amide VI could be isolated. In view of the current interest in this degradation of Δ^{16}-20-ketones to androstane derivatives we considered the use of boron trifluoride as catalyst in this reaction. Recently Hauser and Hoffenberg[2] have used boron trifluoride in the Beckmann rearrange-

(1) G. Rosenkranz, O. Mancera, F. Sondheimer and C. Djerassi, *J. Org. Chem.*, **21**, 520 (1956).

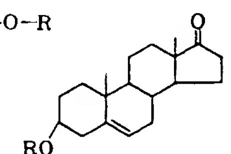
(2) Ch. R. Hauser and D. S. Hoffenberg, THIS JOURNAL, **77**, 4885 (1955).

ment of some acetoximes and obtained in fairly good yield the corresponding amides. Therefore the 20-acetoxime of $\Delta^{5,16}$-pregnadien-3β-ol-20-one-3-acetate (Ib) was prepared and subjected to the action of boron trifluoride etherate in benzene solution and thus there was obtained dehydroepiandrosterone acetate (IIb); but when the rearrangement of the acetoxime Ib or the oxime Ia was carried in acetic anhydride it undertook a different course and two products A and B, were isolated by chromatographic separation. Product A analyzed for $C_{25}H_{34}O_5$, showed m.p. 216–218°, $[\alpha]^{20}D$ −14° ($CHCl_3$). The infrared spectrum showed a band at 5.80 μ (shoulder at 5.88 μ). It formed an oxime and perbenzoic acid treatment afforded a diepoxide, while potassium bicarbonate saponification furnished the free derivative (m.p. 189–191°); product A was identified as 17β-methyl-18-nor-$\Delta^{5,13}$-isopregnadien-3β,16α-diol-20-one 3,16-diacetate (IIIa), which had been obtained by Heusler and Wettstein[3] by rearrangement of 16α,17α-oxido-Δ^5-pregnen-3β-ol-20-one acetate with *p*-toluenesulfonic acid in acetic anhydride. We repeated this reaction and found the substance to be identical with product A by mixed melting point and infrared comparison.[4]

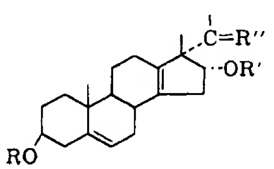
Several derivatives of IIIa were prepared: lithium aluminum hydride reduction afforded the 3,16,-20-triol IIId; the keto group in IIIa was eliminated by hydrogenolysis of the cycloethylene mercaptol IIIf whereupon the diacetate IIIg was obtained; potassium bicarbonate saponification of IIIg yielded IIIh which on Oppenauer oxidation afforded the Δ^4-3-ketone V.



Ia, R = H
b, R = Ac

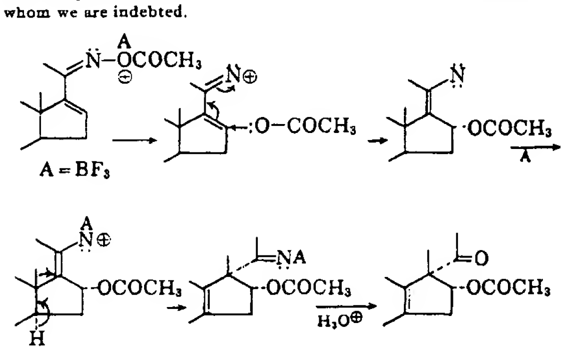


IIa, R = H
b, R = Ac

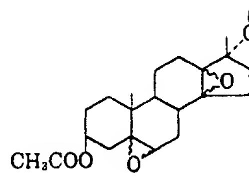


(3) K. Heusler and Wettstein, *Chem. Ber.*, **87**, 1301 (1954).

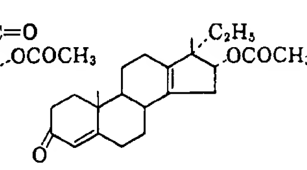
(4) The following mechanism for the formation of product A has been suggested to us by Dr. Gilbert Stork, Columbia University, to whom we are indebted.



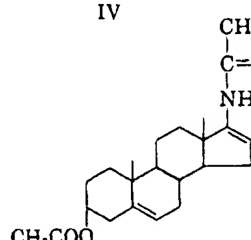
IIIa, R = R' = Ac; R'' = O
b, R = R' = Ac; R'' = N·OH
c, R = R' = H; R'' = O
d, R = R' = H; R'' = <H, OH
e, R = R' = Ac; R'' = <H, OAc
f, R = R' = Ac; R'' = <S–CH₂CH₂–S
g, R = R' = Ac; R'' = H_2
h, R = H; R' = Ac; R'' = H_2



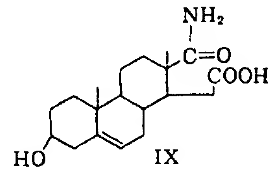
IV



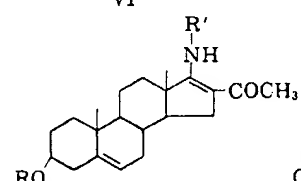
V



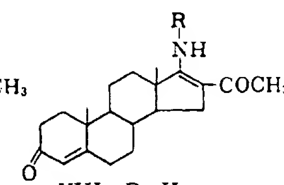
VI



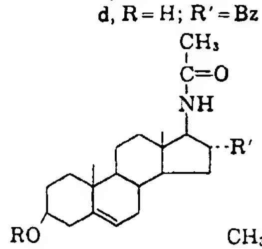
IX



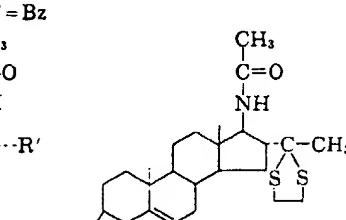
VIIa, R = R' = Ac
b, R = R' = H
c, R = R' = Bz
d, R = H; R' = Bz



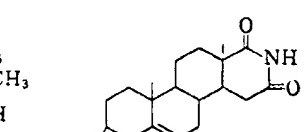
VIIIa, R = H
b, R = Bz



XIa, R = R' = Ac
b, R = H; R' = Ac
c, R = Ac; R' = C_2H_5
d, R = Ac; R' = C(=NOH)–CH_3



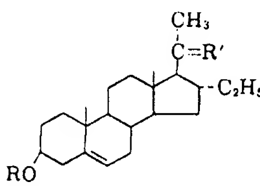
XII



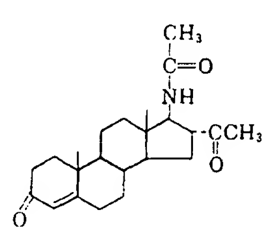
X

Product B contained nitrogen, analyzed for $C_{25}H_{35}O_4N$ and showed m.p. 173–175°. Potassium hydroxide hydrolysis yielded the free compound ($C_{21}H_{31}O_2N$, m.p. 245–246°) which on reacetylation regenerated product B; the free compound gives a blue color with methanolic ferric chloride and a bluish-green precipitate with copper acetate in methanol solution upon addition of a few drops of ammonium hydroxide; the ultraviolet spectrum gave a maximum at 314 mμ (log ε 4.22)

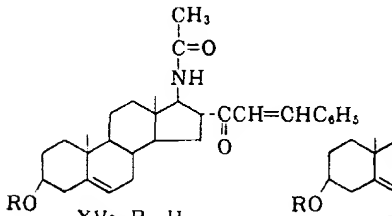
and the infrared curve showed bands at 2.80, 2.90, 3.08, 6.20, 6.35 and 6.70 μ. The formation of a copper chelate and the ultraviolet and infrared spectra strongly indicated the presence of a β-



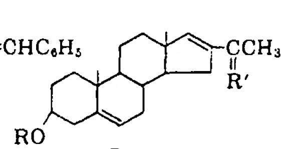
XIIIa, R = H; R' = O
b, R = Ac; R' = O
c, R = Ac; R' = NOH



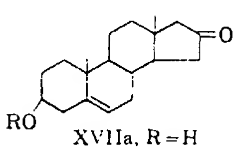
XIV



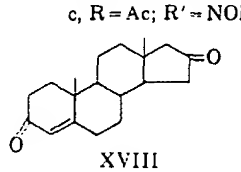
XVa, R = H
b, R = Ac



XVIa, R = H; R' = O
b, R = Ac; R' = O
c, R = Ac; R' = NOH



XVIIa, R = H
b, R = Ac



XVIII

amino-α,β-unsaturated ketone grouping. Combes and Combes,[5a] Ueno and Martell[5b] and Holtzclaw, *et al.*,[6] have described the formation of copper chelates of β-amino-α,β-unsaturated ketones, while Cromwell, *et al.*,[7] have found that the grouping –NH–C=C–CO– shows a maximum in the region of 306–308 mμ, which is very close to the maximum observed in our derivative. The infrared spectra of these amino ketones have been reported by the aforementioned authors (see ref. 5b, 6 and 7), and particularly the bands at 6.20 (hydrogen bonded carbonyl), 6.35 and 6.70 μ (C=C stretching in the hydrogen bonded system) found in our free compound closely correspond to those reported for the β-amino-α,β-unsaturated ketones (see ref. 6).

Further evidence of the presence of the β-amino-α,β-unsaturated ketone grouping and its position arises from the following experiments.

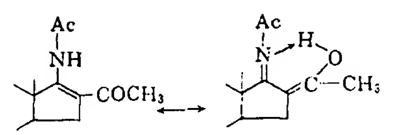
Alkaline hydrogen peroxide oxidation of the free compound furnished an amido acid ($C_{19}H_{29}O_4N$, m.p. 254–256°) which upon acetylation afforded a neutral acetate ($C_{21}H_{29}O_4N$, m.p. 260–262°). These derivatives proved to be identical with the 3β-hydroxy-16,17-seco-Δ^5-androsten-16,17-dioic acid-17-amide (IX) and 3β-acetoxy-16,17-seco-Δ^5-androsten-16,17-imide (X) prepared by Regan and Hayes.[8] The formation of the acid IX would imply an oxidative splitting of a Δ^{16}-double bond with elimination of a 16-acetyl group; therefore a 16-acetyl-17-amino-Δ^{16}-androstene moiety comes into consideration. Another fact which strongly supports this structure is the introduction of an acetyl group into the amide VI by treatment with acetic anhydride in the presence of boron trifluoride etherate which produces the same product B, thus showing that the acetyl group is attached to the 16-position; then, formula VIIa must represent the structure of product B, and VIIb that of the hydrolysis of B. This structure was definitely proved in the following manner.

Selective hydrogenation of the 16-double bond of VIIa with Adams catalyst afforded the dihydro derivative XIa, which formed an oxime (XId) and a benzal derivative XVa; the 16-acetyl side chain of XIa was transformed into the ethyl group XIc by hydrogenolysis of the cycloethylene mercaptol XII.

Product XIc proved to be identical with 16α-ethyl-17-acetylamino-Δ^5-androsten-3β-ol-acetate which was synthesized by addition of ethylmagnesium iodide to $\Delta^{5,16}$-pregnadien-3β-ol-20-one acetate; Marker and Crooks[9] used this method to prepare several 16-alkyl-pregnenolones in which the 16-alkyl group is known to have the α-configuration.[10]

The 16α-ethyl-Δ^5-pregnen-3β-ol-20-one (XIIIa) was converted into the acetate XIIIb and its oxime XIIIc; the latter by Beckmann rearrangement with tosyl chloride in pyridine furnished the acetylamino derivative XIc identical with that obtained by hydrogenolysis of the mercaptol XII.

It is pertinent to note that the hydrogenation of VIIa afforded the dihydro derivative XIa with the 16-acetyl group alpha oriented and it is well known that in the hydrogenation of a Δ^{16}-double bond the hydrogen enters from the rear producing in this way beta oriented groups at positions 16 and 17. It is possible that under the hydrogenation conditions (in presence of acetic acid) equilibration takes place affording the more stable alpha oriented side chain[11] or that equilibration takes place when the cycloethylene mercaptol XII is formed, due to the acidic character of the zinc chloride used as catalyst. It is also of interest to point out that β-amino-α,β-unsaturated ketones (a) are in equilibrium with structure (b)[6] and if the double bond of the latter imine is hydrogenated, then the 16-side



a b

(5) (a) A. Combes and C. Combes, *Bull. soc. chim. France*, [3] **7**, 778 (1892); (b) K. Ueno and A. E. Martell, *J. Phys. Chem.*, **59**, 998 (1955).

(6) H. F. Holtzclaw, Jr., J. Collman and R. M. Alive, THIS JOURNAL, **80**, 1100 (1958).

(7) N. H. Cromwell, F. A. Miller, A. R. Johnson, R. L. Frank and D. J. Wallace, *ibid.*, **71**, 2337 (1949).

(8) B. M. Regan and F. N. Hayes, *ibid.*, **78**, 639 (1956). We are indebted to Dr. Regan for furnishing us with samples of these substances.

(9) R. E. Marker and H. M. Crooks, Jr., *Ibid.* **64**, 1280 (1942).

(10) J. Romo, J. Lepe and M. Romero, *Bol. Inst. de Quim. U.N.A.M.*, No. 2, Vol. IV, 125 (1952).

(11) J. Fajkos and F. Sorm, *Chem. Listy*, **51**, 579 (1959).

chain rearranges to the more stable α-configuration.

Several derivatives of the amino-ketone VIIb were prepared: The dibenzoate VIIc was obtained by treatment of VIIb with benzoyl chloride in pyridine, while Schotten-Baumann benzoylation afforded the monobenzoate VIId; Oppenauer oxidation of VIId yielded the diketone VIIIb; alkaline hydrolysis of VIIIb furnished the amino-diketone VIIIa.

Potassium hydroxide treatment of the dihydroaminoketone XIa only removed the 3-acetyl group yielding XIb, which on Oppenauer oxidation afforded the diketone XIV.

The dihydro aminoketone XIa lost the elements of acetamide on treatment with p-toluenesulfonic acid in acetic anhydride solution, yielding 16-acetyl-$\Delta^{5,16}$-androstadien-3β-ol acetate (XVIb) which on alkaline hydrolysis furnished the free derivative XVIa; these two compounds have been described by Fajkos and Sorm,[11] and the physical constants of our products agree well with those reported (see Experimental part).

The Beckmann rearrangement of 16-acetyl-$\Delta^{5,16}$-androstadien-3β-ol acetate-oxime (XVIc) furnished Δ^5-androstene-3β-ol-16-one acetate (XVIIb) which on saponification yielded the free compound (XVIIa). These substances have been described by Huffman et al.[12] and by Fajkos and Sorm,[13] and our physical constants are in good agreement with those reported by the aforementioned authors.

Acknowledgment.—We wish to thank Dr. G. Rosenkranz of Syntex, S. A. for a generous gift of steroids.

Experimental[14]

Acetoxime of $\Delta^{5,16}$-Pregnadiene-3β-ol-20-one 3-Acetate (Ib).—A solution of 10 g. of the oxime Ia in 30 ml. of anhydrous pyridine and 30 ml. of acetic anhydride was heated on the steam-bath for one hour and then poured into 300 ml. of cold water; the precipitate was collected, washed thoroughly with water and crystallized from chloroform–methanol, thus affording prismatic needles (9.35 g.), m.p. 195–196°; the analytical sample showed m.p. 195–197°, $[\alpha]^{20}D$ −41°.

Anal. Calcd. for $C_{25}H_{35}O_4N$: C, 72.25; H, 8.98; H, 3.37. Found: C, 72.18; H, 8.49; N, 3.47.

Δ^5-Androsten-3β-ol-17-one Acetate (IIb).—To a solution of 2 g. of the acetoxime Ib in benzene, 30 ml., was added 3 ml. of boron trifluoride etherate and the mixture was left for 3 hours at room temperature, diluted then with cold water, washed with 5% sodium carbonate solution and water, dried over anhydrous sodium sulfate and evaporated to a small volume. The ketone IIb crystallized upon addition of hexane (410 mg.), m.p. 164–166°; one crystallization from acetone–hexane raised the m.p. to 168–170°, $[\alpha]^{20}D$ −2°; it gave no depression in mixed m.p. with an authentic specimen and the infrared spectra were identical.

Beckmann Rearrangement of $\Delta^{5,16}$-Pregnadien-3β-ol-20-one 3-Acetate-20-oxime[1] (Ia) with Boron Trifluoride Etherate in acetic anhydride.—A suspension of 50 g. of the oxime Ia in 300 ml. of acetic anhydride was treated with 100 ml. of boron trifluoride etherate in the course of 20 minutes at a temperature below 15° with mechanical stirring; during the addition all of the oxime went into solution. The dark solution was kept for 1 hour between 10–15° and then at room temperature overnight; the mixture was poured into 1300 ml. of ice-water, the oily precipitate was extracted with ether, the ether extract was washed with water, with 5% sodium carbonate solution and again with water, dried over sodium sulfate and evaporated to dryness. The oily residue (46 g.) was dissolved in benzene–hexane 1:1 and chromatographed on 1000 g. of alumina (washed with ethyl acetate) the first fractions eluted with benzene–hexane 1:1 crystallized on evaporation to yield 4.3 g. of product A (IIIa), m.p. 208–212°; the analytical sample showed m.p. 216–218° (prisms from acetone–methanol), $[\alpha]^{20}D$ −14°, λ_{max} 5.8 μ (shoulder at 5.88 μ) (the m.p. of the substance showed no depression in a mixture with a sample obtained by the method of Heusler and Wettstein,[3] and the infrared spectra were identical; this authors report m.p. 213–214.5°, $[\alpha]^{25}D$ −10 ± 4° ($CHCl_3$)).

Anal. Calcd. for $C_{25}H_{34}O_5$: C, 72.43; H, 8.27; O, 19.30. Found: C, 72.53; H, 8.23; O, 19.15.

The last crystalline fractions eluted with benzene–hexane 1:1, with benzene–hexane 2:1, 3:1, 4:1 and with benzene were combined and recrystallized from acetone–methanol, thus furnishing fluffy needles of product β (VIIIb) (39.5 g.), m.p. 168–172°; the analytical sample showed m.p. 173–175° (from acetone–methanol), $[\alpha]^{20}D$ −205°; λ_{max} 310 mμ, log ϵ, 4.10; λ_{max} 5.85, 6.7, 6.40 μ.

Anal. Calcd. for $C_{25}H_{35}O_4N$: C, 72.60; H, 8.53; N, 3.39. Found: C, 72.66; H, 8.58; N, 3.35.

When the reaction was carried out with the acetoxime Ib the same results were obtained.

17β-Methyl-18-nor-$\Delta^{5,13}$-isopregnadiene-3β,16α-diol-20-one 20-oxime-3,16-diacetate (IIIb) was prepared by reaction with hydroxylamine hydrochloride in pyridine and methanol. It showed m.p. 226–228.5° dec., $[\alpha]^{20}D$ −111° (Heusler and Wettstein[3] report m.p. 230–232°).

Anal. Calcd. for $C_{25}H_{35}O_5N$: C, 69.90; H, 8.21; N, 3.26; O, 18.62. Found: C, 70.47; H, 8.23; N, 3.53; O, 18.21.

5,6,13,14-Di-oxido-17β-methyl-18-nor-isopregnan-3β,16α-diol-20-one 3,16-Diacetate.—A solution of 1 g. of the diacetate IIIa in 10 ml. of chloroform was mixed with a solution of 800 mg. of monoperphthalic acid in 20 ml. of ether and the mixture was kept overnight at 5° and then for 4 hours at room temperature, washed with 5% sodium carbonate solution and water, dried and evaporated to dryness. The residue crystallized upon the addition of methanol in the form of small plates (490 mg.), m.p. 235–236°; recrystallization from acetone–ether raised the m.p. to 241–242°, $[\alpha]^{20}D$ −64°, λ_{max} 5.80 μ (shoulder at 5.87 μ).

Anal. Calcd. for $C_{25}H_{34}O_7$: C, 67.24; H, 7.67; O, 25.09. Found: C, 67.59; H, 7.70; O, 25.10.

17β-Methyl-18-nor-$\Delta^{5,13}$-isopregnadien-3β,16α-diol-20-one (IIIc).—A solution of 600 mg. of the diacetate IIIa in 30 ml. of methanol was mixed with 600 mg. of potassium bicarbonate in 10 ml. of water and the mixture was refluxed for 1 hour, diluted with water and extracted with chloroform; the chloroform extract was washed with water and evaporated to dryness in vacuum. Crystallization of the residue from acetone–hexane yielded small prisms (360 mg.), m.p. 176–181°; further recrystallizations from acetone–ether raised the m.p. to 189–191°, $[\alpha]^{20}D$ −123°; λ_{max} 2.8, 2.95, 5.9 μ (reported[3] m.p. 189–191°, $[\alpha]^{20}D$ −131 ± 4° ($CHCl_3$)).

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 75.84; H, 9.12.

17β-Methyl-18-nor-$\Delta^{5,13}$-isopregnadien-3β,16α,20-triol (IIId).—One gram of the diacetate IIIa was dissolved in 50 ml. of anhydrous tetrahydrofuran and slowly added to a suspension of 800 mg. of lithium aluminum hydride in 150 ml. of ether and the mixture was refluxed for 1 hour; the excess of hydride was decomposed with a few drops of ethyl acetate, then poured into water and acidified with 10% sulfuric acid; the precipitate was extracted with ethyl acetate, washed with water, concentrated to small volume and crystallized by the addition of ether, thus yielding 640 mg., m.p. 183–185°; the analytical sample was obtained as large needles by crystallization from benzene-hexane, m.p. 202–204°, $[\alpha]$ −207°, λ_{max} 2.9–3.0 μ.

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.98; H, 9.76.

(12) M. N. Huffman, M. H. Lott and A. Tillotson, *J. Biol. Chem.*, **218**, 567 (1956).

(13) J. Fajkos and F. Sorm, *Chem. Listy*, **50**, 282 (1956).

(14) Melting points are uncorrected. Rotations were determined at 20° in chloroform, unless noted otherwise. The ultraviolet absorption spectra were determined in 95% ethanol solution in a Beckman DK2 spectrophotometer. The infrared spectra were determined in chloroform solution on a Perkin-Elmer double-beam spectrophotometer (unless stated otherwise). The microanalyses were performed by Dr. Franz Pascher, Bonn, Germany.

The triacetate (IIIe) (acetic anhydride and pyridine at room temperature overnight) showed m.p. 121–122° (small prisms from hexane), $[\alpha]^{20}D$ −149.5°, λ_{max} 5.85 μ.

Anal. Calcd. for $C_{27}H_{38}O_6$: C, 70.71; H, 8.35; O, 20.94. Found: C, 70.83; H, 8.36; O, 21.17.

Cycloethylene Mercaptol (IIIf).—A solution of 1 g. of the diacetate IIIa and 1 g. of ethanedithiol in 50 ml. of acetic acid was treated with 1 ml. of a saturated solution of hydrogen bromide in glacial acetic acid and the mixture was kept for 3 hours at room temperature, poured into 300 ml. of cold water and rapidly extracted with ether; the organic layer was washed with 5% sodium hydroxide solution and water, evaporated to dryness and the oily residue crystallized from methanol, thus giving 510 mg. of mercaptol in the form of needles, m.p. 137–140°; the analytical sample showed m.p. 149–151° (from acetone–methanol), $[\alpha]^{20}D$ −108°.

Anal. Calcd. for $C_{27}H_{38}O_4S_2$: C, 66.10; H, 7.81; S, 13.04; O, 13.04. Found: C, 65.87; H, 8.07; S, 12.78; O, 13.19.

17β-Methyl-17α-ethyl-18-nor-$\Delta^{5,13}$-androstadien-3β,16α-diol 3,16-Diacetate (IIIg).—The mercaptol IIIf (600 mg.) was hydrogenolyzed in 125 ml. of ethanol with 5 g. of Raney nickel[15] by refluxing for 3 hours, the nickel was removed by filtration and the solution evaporated to dryness; crystallization from acetone–methanol afforded small plates (380 mg.), m.p. 118–120°; further recrystallizations from acetone–methanol raised the m.p. to 123–124°, $[\alpha]^{20}D$ −176°, λ_{max} 5.85 μ.

Anal. Calcd. for $C_{25}H_{36}O_4$: C, 74.96; H, 9.06; O, 15.98. Found: C, 75.26; H, 9.00; O, 16.19.

17β-Methyl-17α-ethyl-18-nor-$\Delta^{4,13}$-androstadien-16α-ol-3-one acetate (V).—The diacetate IIIg (1 g.) in methanol (40 ml.) was saponified with sodium hydroxide (1 g.) in water (10 ml.) by refluxing for 1 hour, the solution was diluted with water and extracted with ether, the ethereal extract was washed with water and evaporated to dryness. The oily residue was dissolved in 40 ml. of toluene and 10 ml. of cyclohexanone, 10 ml. was distilled to eliminate moisture; a solution of 800 mg. of aluminum isopropylate in 10 ml. of toluene then was added and the mixture refluxed for 1 hour, diluted with benzene and washed with 5% hydrochloric acid and water and the organic solvents were removed by steam distillation. The oily residue was extracted with ether, dried over sodium sulfate, evaporated to dryness (890 mg. of residue), dissolved in hexane and chromatographed on washed alumina. The crystalline fractions eluted with hexane were combined and recrystallized from acetone–hexane, m.p. 144–146° (240 mg.). The analytical sample showed m.p. 147–148° (prismatic needles from acetone–hexane), $[\alpha]^{20}D$ −53.6°; λ_{max} 238–240 mμ, log ε, 4.17; λ_{max} 5.85, 6.05 μ.

Anal. Calcd. for $C_{23}H_{32}O_3$: C, 77.49; H, 9.05; O, 13.46. Found: C, 77.41; H, 9.09; O, 13.73.

16-Acetyl-17-amino-$\Delta^{5,16}$-androstadien-3β-ol (VIIb).—A mixture of 4.7 g. of the diacetate VIIa and 60 ml. of methanol was treated with a solution of 4 g. of potassium hydroxide in 10 ml. of water and refluxed for 1 hour; the solution was concentrated to half its volume, diluted with water and the precipitate collected; crystallization from methanol-acetone furnished 2.46 g., m.p. 242–245°, and further recrystallizations from methanol–acetone raised the m.p. to 245–246°, $[\alpha]^{20}D$ −136°; λ_{max} 314 mμ, log ε, 4.22; λ_{max} 2.80, 2.90, 3.08, 6.20, 6.35 and 6.70 μ.

A methanolic solution of the product gives a blue color with aqueous ferric chloride and gives a bluish-green precipitate with a copper acetate–ammonic solution.

Anal. Calcd. for $C_{21}H_{31}O_2N$: C, 76.55; H, 9.48; N, 4.25; O, 9.71. Found: C, 76.39; H, 9.50; N, 4.39; O, 10.05.

Reacetylation with acetic anhydride and pyridine regenerates the diacetate VIIa.

3β-Hydroxy-16,17-seco-Δ^5-androsten-16,17-dioic Acid 17-Amide (IX).—A solution of the amino-ketone VIIb (3.38 g.) and potassium hydroxide (4 g.) in methanol (120 ml.) and water (10 ml.) was mixed with 10 ml. of 35% hydrogen peroxide and the solution was refluxed 20 minutes; 10 ml. more of hydrogen peroxide was added and the reflux prolonged for 20 minutes more; the solution was concentrated to a small volume, diluted with water, filtered and acidified with 10% hydrochloric acid solution. The precipitate was collected, washed with water and crystallized from chloroform-methanol, furnishing needles (840 mg.), m.p. 235–239°. The analytical sample showed m.p. 254–256° dec., $[\alpha]^{20}D$ −68° (dioxane); λ_{max} (Nujol) 2.95, 5.87, 6.1 μ. (This product showed no depression in mixed m.p. with an authentic sample; Regan[8] reported m.p. 255–257° dec.)

Anal. Calcd. for $C_{19}H_{29}O_4$: C, 68.03; H, 8.71; N, 4.18; O, 19.08. Found: C, 68.26; H, 8.79; N, 4.57; O, 19.19.

16,17-Seco-Δ^5-androsten-3β-ol-16,17-imide Acetate (X).—A mixture of 300 mg. of the acid IX, 2 ml. of acetic anhydride and 2 ml. of pyridine were heated on the steam-bath for 1 hour, poured into water and the precipitate collected; crystallization from methanol afforded needles (210 mg.), m.p. 260–262°, $[\alpha]^{20}D$ −138°; λ_{max} 3.0 (NH), 5.82, 5.9 μ. The mixed m.p. with an authentic specimen[8] showed no depression and the infrared spectra were identical).

Anal. Calcd. for $C_{21}H_{29}O_4N$: C, 70.17; H, 8.13; O, 17.80; N, 3.90. Found: C, 70.59; H, 8.19; O, 17.65; N, 3.72.

Treatment of 17-Acetylamino-$\Delta^{5,16}$-androstadien-3β-ol Acetate (VI) with Acetic Anhydride and Boron Trifluoride Etherate.—A solution of the acetylamino derivative VI (2.4 g.) in acetic anhydride (20 ml.) was treated with 4 ml. of boron trifluoride etherate and kept for 1 hour at a temperature, below 15°, poured into 200 ml. of cold water and worked up as in the isolation of product B. Crystallization from acetone–hexane yielded fluffy needles (1.915 g.), m.p. 170–172°. This product showed no depression in mixed m.p. with the acetyl aminoketone VIIa and the infrared spectra were superimposable.

16α-Acetyl-17-acetylamino-Δ^5-androsten-3β-ol Acetate (XIa).—The acetylaminoketone VIIa (1 g.) in 80 ml. of ethyl acetate and 20 ml. of acetic acid was hydrogenated with 100 mg. of Adams catalyst until 1 mole of hydrogen was absorbed (3 hours); the catalyst was removed by filtration and the solution washed with dilute sodium carbonate and water, evaporated to a small volume and crystallized by the addition of ether (prisms (835 mg.), m.p. 226–230°); further crystallizations from methanol–ether raised the m.p. 239–241°, $[\alpha]^{20}D$ −77°; λ_{max} 2.95, 5.83, 6.0 μ.

Anal. Calcd. for $C_{25}H_{37}O_4N$: C, 72.25; H, 8.98; O, 15.40; N, 3.37. Found: C, 72.40; H, 8.50; O, 15.34; N, 3.25.

The oxime XId showed m.p. 298–299° (from chloroform–methanol), $[\alpha]^{20}D$ −81°.

Anal. Calcd. for $C_{25}H_{38}O_4N_2$: C, 69.73; H, 8.90; O, 14.86; N, 6.51. Found: C, 69.95; H, 8.86; O, 14.91; N, 6.43.

16α-Acetyl-17-acetylamino-Δ^5-androsten-3β-ol (XIb).—The diacetate XIa (600 mg.) was saponified with 30 ml. of methanol and 400 mg. of potassium hydroxide by refluxing for 1 hour. The mixture was diluted with water, extracted with chloroform and the extract washed with water and concentrated. Upon the addition of ether there crystallized the product as plates (450 mg.), m.p. 256–258°. The analytical sample showed m.p. 265–267° (from methanol–ether), $[\alpha]^{20}D$ −91° (dioxane).

Anal. Calcd. for $C_{23}H_{35}O_3N$: C, 73.95; H, 9.45; O, 12.85; N, 3.75. Found: C, 74.11; H, 9.42; O, 13.08; N, 3.78.

16α-Acetyl-17-acetylamino-Δ^4-androsten-3-one (XIV).—The monoacetate XIb (880 mg.) was dissolved in 50 ml. of toluene and 10 ml. of cyclohexanone, 10 ml. of solvent was distilled to remove moisture and a solution of 400 mg. of aluminum isopropylate in 10 ml. of toluene was added. The mixture was refluxed for 1 hour, then washed with dilute hydrochloric acid and the volatile components were removed by steam distillation. The semi-solid residue was extracted with chloroform, washed with water and evaporated to dryness; crystallization from acetone–ether afforded shiny plates (580 mg.), m.p. 205–207°. The analytical sample showed m.p. 210–212° (from acetone–ether), $[\alpha]^{20}D$ +58°; λ_{max} 239–240 mμ, log ε, 4.09; λ_{max} 2.95, 5.9 and 6.05 μ.

Anal. Calcd. for $C_{23}H_{33}O_3N$: C, 74.36; H, 8.95; O, 12.92; N, 3.77. Found: C, 74.00; H, 8.87; O, 13.45; N, 3.81.

Benzal Derivative of 16α-Acetyl-17-acetylamino-Δ^5-an-

(15) Prepared following the method described by R. Mozingo, D. E. Wolfrom, S. A. Harris and K. Folkers, This Journal, 65, 1013 (1943).

drosten-3β-ol (XVa).—Benzaldehyde (0.1 ml.) was added to a solution of the diacetate XIa (200 mg.) and potassium hydroxide (300 mg.) in methanol (10 ml.). The mixture was kept at room temperature overnight, whereupon long needles crystallized which were collected and washed thoroughly with methanol, m.p. 313–315° (210 mg.). The analytical sample showed m.p. 314–316° (from acetic acid–methanol).

Anal. Calcd. for $C_{30}H_{39}O_3N$: C, 78.05; H, 8.52; N, 3.03. Found: C, 77.78; H, 8.48; N, 2.83.

The acetate showed m.p. 304–306° (small needles from pyridine); λ_{max} 290–292 mμ, log ε 4.25.

Anal. Calcd. for $C_{32}H_{41}O_4N$: C, 76.31; H, 8.21; N, 2.78. Found: C, 76.63; H, 8.21; N, 2.82.

Cycloethylene Mercaptol of 16α-Acetyl-17-acetylamino-Δ^5-androsten-3β-ol Acetate (XII).—The diacetate XIa (1.5 g.) and ethanedithiol (3 ml.) were dissolved in anhydrous dioxane (20 ml.) and freshly fused and finely powdered zinc chloride (15 g.) and anhydrous sodium sulfate (10 g.) were added. The semi-solid mixture was kept at room temperature overnight, poured into water and extracted with chloroform; the organic layer was washed with water, dried over anhydrous sodium sulfate and evaporated to dryness. Crystallization of the residue from ethyl acetate–hexane afforded needles (1.62 g.), m.p. 191–193°; further crystallizations raised the m.p. to 194–195° (from acetone–ether), $[\alpha]^{20}D$ −78°.

Anal. Calcd. for $C_{27}H_{41}O_3S_2N$: C, 65.96; H, 8.41; O, 9.77; S, 13.01; N, 2.85. Found: C, 65.71; H, 8.24; O, 9.46; S, 12.80; N, 2.73.

16α-Ethyl-17-acetylamino-Δ^5-androsten-3β-ol Acetate (XIc). By Hydrogenolysis of the Cycloethylene-mercaptol XII.—To a solution of the mercaptol XII (1.5 g.) in 200 ml. of ethanol there was added 15 g. of Raney nickel and the suspension was refluxed for 3 hours, filtered and the solvent concentrated to small volume. There separated shiny plates, m.p. 183–185°, which upon further crystallizations from acetone–hexane showed m.p. 195–196°, $[\alpha]^{20}D$ −120.3°; λ_{max} 2.95, 5.83, 6.03 μ.

Anal. Calcd. for $C_{25}H_{39}O_3N$: C, 74.77; H, 9.76; O, 11.95; N, 3.49. Found: C, 74.52; H, 9.71; O, 12.21; N, 3.46.

16α-Ethyl-Δ^5-pregnen-3β-ol-20-one Acetate (XIIIb).—From a solution of 3 g. of $\Delta^{5,16}$-pregnadien-3β-ol-20-one acetate in 100 ml. of benzene there was distilled 20 ml. to remove moisture and a solution of ethylmagnesium iodide (prepared from 12 g. of ethyl iodide) in 25 ml. of ether was added and the mixture refluxed for 3 hours, poured into cold water and acidified with diluted hydrochloric acid. The organic layer was diluted with 100 ml. of ether and washed with water, dried over anhydrous sodium sulfate and evaporated to dryness (weight 3.05 g.). The ketonic and nonketonic fractions were separated with Girard T reagent. The ketonic fractions weighed 2.2 g. and were acetylated by heating for 1 hour on the steam-bath with 6 ml. of acetic anhydride and 6 ml. of pyridine, poured into water and the semi-solid precipitate was extracted with ether; the ether extract was washed with dilute hydrochloric acid, sodium carbonate solution and water and then evaporated to dryness. Crystallization from methanol furnished shiny plates, m.p. 120–121° (1.88 g.). The analytical sample showed m.p. 123–125°, $[\alpha]^{20}D$ −78° λ_{max} 5.85 μ.

Anal. Calcd. for $C_{25}H_{38}O_3$: C, 77.67; H, 9.91; O, 12.42. Found: C, 77.32; H, 9.90; O, 12.81.

The oxime (prepared by the usual method: hydroxylamine hydrochloride and pyridine) showed m.p. 156–158°, $[\alpha]^{20}D$ −79°.

Anal. Calcd. for $C_{25}H_{39}O_3N$: C, 74.77; H, 9.79; O, 11.95; N, 3.49. Found: C, 75.16; H, 9.78; O, 11.71; N, 3.31.

16α-Ethyl-17-acetylamino-Δ^5-androsten-3β-ol Acetate (XIc). By Beckmann Rearrangement of the Oxime XIIIc.—Tosyl chloride (2 g.) was added to a solution of the oxime XIIIc (2 g.) in anhydrous pyridine (40 ml.), the mixture was kept at room temperature for 3 hours, poured into 200 ml. of 10% sulfuric acid solution and extracted with ether, washed with water and concentrated to a small volume, thus giving plates (1.36 g.), m.p. 194–196°; further crystallization from acetone raised the m.p. to 196–197°, $[\alpha]$ −120.8° (the mixed m.p. with the product obtained by hydrogenolysis of the mercaptol XII showed no depression and the infrared spectra were identical).

16-Acetyl-17-benzoylamino-$\Delta^{5,16}$-androstadien-3β-ol 3-benzoate (VIIc) (prepared with benzoyl chloride and pyridine, 1 hour on the steam-bath) showed m.p. 279–281° (needles from acetone), $[\alpha]^{20}D$ −44°.

Anal. Calcd. for $C_{35}H_{39}O_4N$: C, 78.18; H, 7.31; O, 11.90; N, 2.61. Found: C, 78.75; H, 7.43; O, 11.31; N, 2.51.

16-Acetyl-17-benzoylamino-$\Delta^{5,16}$-androstadien-3β-ol (VIId).—The aminoketone VIb (1 g.) was suspended in a solution of potassium hydroxide (4 g.) in water (40 ml.), benzoyl chloride (2 g.) was added, the flask was stoppered and shaken for 20 minutes. It was then diluted with water and the precipitate collected, washed thoroughly with water and crystallized from acetone–ether, thus furnishing prisms (810 mg.), m.p. 186–192°; further crystallizations from acetone–ether raised the m.p. to 218–220°, $[\alpha]^{20}D$ −51°.

Anal. Calcd. for $C_{28}H_{35}O_3N$: C, 77.56; H, 8.14; O, 11.07; N, 3.23. Found: C, 77.38; H, 8.27; O, 10.69; N, 3.19.

16-Acetyl-17-benzoylamino-$\Delta^{4,16}$-androstadien-3-one (VIIIb).—The monobenzoate VIId (750 mg.) was dissolved in toluene (50 ml.) and cyclohexanone (10 ml.), a few ml. of the solution was distilled to remove moisture and then a solution of aluminum isopropylate (500 mg.) in toluene (5 ml.) was added. The mixture was refluxed for 1 hour and then the product was isolated as in previous cases. Crystallization from chloroform–ether furnished fluffy needles (520 mg.), m.p. 220–222°. The analytical sample showed m.p. 221–222° (from chloroform–ether), $[\alpha]^{20}D$ +94°; λ_{max} 242, 326–330 mμ, log ε, 4.47, 4.10; λ_{max} 2.95 (weak), 6.03, 6.30, 6.40 μ.

Anal. Calcd. for $C_{28}H_{33}O_3N$: C, 77.92; H, 7.71; O, 11.12; N, 3.25. Found: C, 77.87; H, 7.89; O, 11.33; N, 2.69.

16-Acetyl-17-amino-$\Delta^{4,16}$-androstadien-3-one (VIIIa).—The benzoate VIIIb (400 mg.) was dissolved in a solution of potassium hydroxide (400 mg.) in methanol (30 ml.) and refluxed for 1 hour, diluted with water and the precipitate extracted with chloroform; the extract was washed with water, evaporated to dryness and the residue crystallized from acetone–ether, yielding prisms (280 mg.), m.p. 123–125°; the analytical sample showed m.p. 123–125°, resolidified and remelts at 161–162° (from acetone–ether). The methanolic solution gives a blue color with ferric chloride; $[\alpha]^{20}D$ −8°; λ_{max} 238–240 mμ, log ε, 4.22; λ_{max} 2.92, 3.08, 6.05, 6.20, 6.35 μ.

Anal. Calcd. for $C_{21}H_{29}O_2N$: C, 77.02; H, 8.93; O, 9.77; N, 4.28. Found: C, 76.69; H, 8.77; O, 9.07; N 4.29.

16-Acetyl-$\Delta^{5,16}$-androstadien-3β-ol Acetate (XVIb).—The aminoketone XIa (300 mg.) and *p*-toluenesulfonic acid (40 mg.) were dissolved in acetic anhydride (8 ml.) (1 drop of pyridine was added) the solution was refluxed for 1.5 hours and then poured into cold water. When the acetic anhydride had hydrolyzed, the precipitate was extracted with ether, the organic layer washed with dilute sodium carbonate solution and water. Upon concentration of the extract there crystallized 70 mg. of unreacted product. The mother liquor was evaporated to dryness and the residue crystallized from methanol, thus yielding needles (105 mg.), m.p. 191–195°; further crystallization from acetone–methanol raised the m.p. 201–203°, $[\alpha]^{20}D$ −132°; λ_{max} 240–242 mμ, log ε, 4.07; λ_{max} 5.73, 6.05, 6.3 (weak) μ [reported[11] m.p. 200–201°, $[\alpha]^{20}D$ −130° ($CHCl_3$); λ_{max} 240 mμ; ε 9780.]

Anal. Calcd. for $C_{23}H_{32}O_3$: C, 77.49; H, 9.05; O, 13.46. Found: C, 77.40; H, 8.79; O, 13.82.

The oxime XVIc showed m.p. 212–215° dec. (from chloroform–methanol), $[\alpha]^{20}D$ −128°.

Anal. Calcd. for $C_{23}H_{33}O_3N$: C, 74.36; H, 8.95; O, 12.92; N, 3.77. Found: C, 73.98; H, 8.99; O, 13.27; N, 4.12.

16-Acetyl-$\Delta^{5,16}$-androstadien-3β-ol (XVIa).—The acetate XVIb (1 g.) in methanol (30 ml.) was mixed with a solution of potassium bicarbonate (1 g.) in water (10 ml.) and refluxed for 1 hour. The solution was concentrated to a small volume, diluted with water and the precipitate was collected. Crystallization from acetone–ether afforded

prisms (540 mg.), m.p. 170–172°; the analytical sample showed m.p. 177–179° (from acetone–ether), $[\alpha]^{20}D$ −115°; λ_{max} 240–242 mμ, log ε, 4.16; λ_{max} 2.95, 6.05, 6.30 μ (reported[11] m.p. 181–182°, $[\alpha]^{20}D$ −132°).

Anal. Calcd. for $C_{21}H_{30}O_2$: C, 80.21; H, 9.62; O, 10.18. Found: C, 79.98; H, 9.53; O, 10.11.

Δ⁶-Androsten-3β-ol-16-one Acetate (XVIIb).—The solution of the oxime XVIc (1.3 g.) and tosyl chloride (1.3 g.) in anhydrous pyridine (30 ml.) was treated as in the previous case. Crystallization from pentane furnished needles (510 mg.), m.p. 129–130.5°, and 220 mg. more from the mother liquors, m.p. 121–123°. The analytical sample showed m.p. 129.5–130.5°, $[\alpha]^{20}D$ −222°, λ_{max} 5.82 μ. (Huffman, *et al.*,[12] reported m.p. 127.5–128°; Fajkos and Sorm,[13] m.p. 134–135°, $[\alpha]^{20}D$ −238° ($CHCl_3$).)

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15; O, 14.53. Found: C, 76.69; H, 9.13; O, 14.28.

The free alcohol XVIIa showed m.p. 163–165°, $[\alpha]^{20}D$ −235°; λ_{max} 3, 5.78 μ (reported by Huffman, *et al.*,[12] m.p. 163.5–165°, $[\alpha]^{20}D$ −242° in $CHCl_3$; Fajkos and Sorm,[13] m.p. 168–169°, $[\alpha]^{20}D$ −255° in $CHCl_3$).

Anal. Calcd. for $C_{19}H_{28}O_2$: C, 79.12; H, 9.79; O, 11.10. Found: C, 79.42; H, 9.82; O, 11.29.

Δ⁴-Androstene-3,16-dione (XVIII).—Oppenauer oxidation of the free alcohol XVIIa (750 mg.) furnished thick prisms (410 mg.), m.p. 152–153° (from acetone–hexane), $[\alpha]^{20}D$ −90°, λ_{max} 240 mμ, log ε, 4.22; λ_{max} 5.78, 6.05 μ (reported[13] m.p. 152–153°, $[\alpha]^{20}D$ −90.5° in $CHCl_3$).

Anal. Calcd. for $C_{19}H_{26}O_2$: C, 79.68; H, 9.15; O, 11.17. Found: C, 79.48; H, 9.13; O, 11.45.

MÉXICO, D. F., MEXICO

[CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE]

Cellulose Ion Exchange and Rotatory Dispersion Studies with the Bacitracin Polypeptides

BY WM. KONIGSBERG AND L. C. CRAIG

RECEIVED DECEMBER 29, 1958

Bacitracin has been shown to undergo a change at a *p*H of 4 or less which has been traced to an epimerization of the terminal isoleucine residue. The resulting stereoisomers showing different antibiotic activity have been separated by cellulose ion exchange chromatography. Anomalous rotatory dispersion behavior has been found which has been shown to be connected with the thiazoline complex. Data have been obtained which suggest an interaction of the thiazoline grouping with a ring peptide linkage.

In spite of the careful work done in a number of laboratories[1–3] on the fractionation of the mixture of polypeptides present in commerical bacitracin, a recent paper[4] has indicated that a very subtle type of heterogeneity still has persisted even in bacitracin A. The heterogeneity was correlated with small shifts in optical activity and was of particular importance to the antibiotic potency.

The sequential formula I proposed independently in two laboratories[5,6] contains a thiazoline ring structure which is unique in naturally occurring peptides. Recently, however, the possibility of such a ring being present[7,8] as one of the energy rich states in certain natural products of considerable biological interest has been raised. No relationship of these substances to bacitracin, other than the possible common occurrence of the thiazoline ring, has been shown. Irrespective of this possibility, a substance with formula I would be expected to undergo transformation easily, as indeed has been found to be the case with bacitracin A. It now appears very likely that a type of tautomeric or resonating system of linkages exists in bacitracin A which has not thus far been clearly elucidated. Chemical procedures such as those involving direct hydrolysis with acid may act by a preliminary rearrangement or splitting as is now known to happen with the thiazoline ring and thus fail to reveal the more subtle aspects of the structure or structures. For example, there is reason to believe[6,9] that the phenylalanine residue is in some way connected to the isoleucine-cysteine residues which form the thiazoline ring system. Yet a rigid structural formula does not permit a covalent bond except by having two nitrogens linked to the carbon which emerges on hydrolysis as the carbonyl of the phenylalanyl residue. Although not accepted ordinarily in peptide chemistry as a valid linkage, unusual configurations such as those found in the ergot peptides[10,11] apparently can stabilize the linkage to the extent that it becomes a significant member of the various resonance and tautomeric forms of the amide linkage.

Often the interpretation of complex and labile structures is greatly assisted by spectroscopic methods. Indeed ultraviolet absorption measurements have been very helpful[9] in supporting the thesis of the thiazoline ring in bacitracin A. Although infrared studies have been made in this Laboratory, clear interpretation has been hindered by the multiplicity of groups present which could contribute to specific bands.

Recently the introduction of the Rudolph spectrophotometric polarimeter has considerably facilitated the use of rotatory dispersion in the study of natural products. Its application to the bacitracin polypeptides together with new absorption

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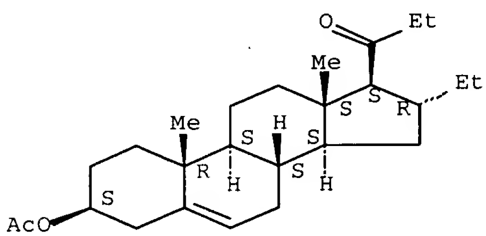
(2) G. G. F. Newton and E. P. Abraham, *Biochem. J.*, **53**, 597 (1953).
(3) J. Porath, *Acta Chem. Scand.*, **8**, 1813 (1954).
(4) L. C. Craig and Wm. Konigsberg, *J. Org. Chem.*, **22**, 1345 (1957).
(5) W. Hausmann, J. R. Weisiger and L. C. Craig, THIS JOURNAL, **77**, 723 (1955).
(6) I. M. Lockhart and E. P. Abraham, *Biochem. J.*, **58**, 633 (1954).
(7) R. E. Basford and F. M. Huennekens, THIS JOURNAL, **77**, 3878 (1955).
(8) D. Cavallini, B. Mondovi and C. DeMarco, *Experientia*, **13**, 436 (1957).
(9) J. R. Weisiger, W. Hausmann and L. C. Craig, THIS JOURNAL, **77**, 3123 (1955).
(10) W. A. Jacobs and L. C. Craig, *J. Biol. Chem.*, **110**, 521 (1935).
(11) A. Stoll, A. Hoffman and Th. Petrzilka, *Helv. Chim. Acta*, 1544 (1951).

Compound (VII)

CAS Registry Record:

RN 16321-62-3 REGISTRY
ED Entered STN: 16 Nov 1984
CN 1-Propanone, 1-[(3β,16α,17β)-16-ethyl-3-(acetyloxy)androst-5-en-17-yl]- (9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN 1-Propanone, 1-(16α-ethyl-3β-hydroxyandrost-5-en-17β-yl)-, acetate (8CI)
FS STEREOSEARCH
MF C26 H40 O3
LC STN Files: CA, CAPLUS, USPATFULL

Absolute stereochemistry.



2 REFERENCES IN FILE CA (1907 TO DATE)
2 REFERENCES IN FILE CAPLUS (1907 TO DATE)

Refs in Chem. Abs.:

L36 ANSWER 1 OF 2 HCA COPYRIGHT 2006 ACS on STN
AN 136:257280 HCA Full-text
TI Methods and compositions that affect melanogenesis
IN Orlow, Seth J.; Hall, Andrea; Manga, Prashiela
PA USA
SO U.S. Pat. Appl. Publ., 63 pp., Cont.-in-part of U. S. Ser. No. 599,487. CODEN: USXXCO
DT Patent
LA English
FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2002034772	A1	20020321	US 2001-827428	20010406
	WO 2002098347	A2	20021212	WO 2002-US11067	20020408
	WO 2002098347	A3	20030501		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
	EP 1383474	A2	20040128	EP 2002-776548	20020408

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
	JP 2004529975	T2	20040930	JP 2003-501389	20020408

TW 235659	B1	20050711	TW 2002-91107018	20020408
US 2004175767	A1	20040909	US 2004-758335	20040115
US 2006188953	A1	20060824	US 2006-408108	20060420
PRAI US 1999-141563P	P	19990629		
US 2000-599487	A2	20000623		
US 2001-827428	A	20010406		
WO 2002-US11067	W	20020408		
US 2004-758335	A3	20040115		

AB The invention provides methods of screening for compds. that affect melanogenesis and the function of P protein in organisms, cells, or cell-free systems. The invention further relates to pharmacol. and cosmetic uses of methods of inhibiting melanogenesis, methods of activating melanogenesis, and compds. and pharmacol. compns. useful for the inhibition or activation of melanogenesis and, therefore, for lightening or darkening the pigmentation of cells and tissue, i.e., skin.

L36 ANSWER 2 OF 2 HCA COPYRIGHT 2006 ACS on STN
AN 67:117077 HCA Full-text
TI 16α-Alkyl-or 16α-aryl-17β-acyl derivatives of androstene
IN Maksimov, V. I.; Lur'i, F. A.; Morozova, L. S.
PA Ordzhonikidze, S., All-Union Scientific-Research Chemical-Pharmaceutical Institute
SO U.S.S.R.
From: Izobret., Prom. Obraztsy, Tovarnye Znaki 1966, 43(22), 37.
CODEN: URXXAF
DT Patent
LA Russian
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	**SU 188492**		19661101	SU	19650701

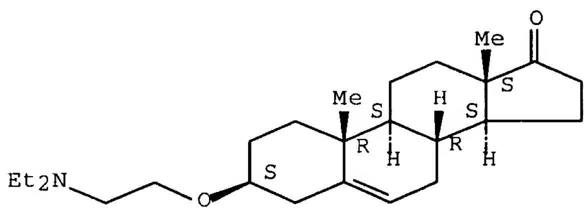
GI For diagram(s), see printed CA Issue.
AB The title compds. of the general formula I where the double bond is located between C-4 and C-5 or C-5 and C-6, R is O or H, β-OH, R1 is an alkyl or an aryl, are prepared by treating 17-cyanoandrosta-5,16-dien-3- ol with excess alkylmagnesium halide in an organic solvent medium, e.g. anisole, at 60-85°, in a current of N. The resulting product is subjected to saponification and oxidation

Compound (VIII)

CAS Registry Record:

RN 2855-62-1 REGISTRY
ED Entered STN: 16 Nov 1984
CN Androst-5-en-17-one, 3-[2-(diethylamino)ethoxy]-, (3β)- (9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN Androst-5-en-17-one, 3β-[2-(diethylamino)ethoxy]- (7CI)
OTHER NAMES:
CN LS 3360
FS STEREOSEARCH
MF C25 H41 N O2
CI COM
LC STN Files: BEILSTEIN*, CA, CAOLD, CAPLUS, USPATFULL
(*File contains numerically searchable property data)

Absolute stereochemistry.



9 REFERENCES IN FILE CA (1907 TO DATE)
9 REFERENCES IN FILE CAPLUS (1907 TO DATE)
3 REFERENCES IN FILE CAOLD (PRIOR TO 1967)

Oldest Refs in Chem. Abs.:

L27 ANSWER 3 OF 3 CAOLD COPYRIGHT 2006 ACS on STN
AN CA58:11781e CAOLD
TI cholesterol biosynthesis - (IV) reduction of lanosterol to 24,25-dihydrolanosterol by rat liver homogenates
AU Avigan, Joel; Goodman, D. S.; Steinberg, D.
IT 79-62-9 2855-62-1 107744-23-0

with toluidine blue was less for operated than for normal rats. — A. Hepburn

Cholesterol biosynthesis. IV. luction of lanosterol to 24,25-dihydrolanosterol by rat livei ...ogenates. Joel Avigan, DeWitt S. Goodman, and Daniel Steinberg (U.S. Public Health Serv., Bethesda, Md.). *J. Biol. Chem.* **238**, 1283-8(1963); cf. *CA* **56**, 10753*i*. The anaerobic redn. of labeled lanosterol, biosynthetically prepd. from 2-C^{14}-mevalonic acid, to 24,25-dihydrolanosterol has been demonstrated with rat liver homogenates. Enzymic activity was assocd. with cell particles, mostly with microsomes, and required reduced triphosphopyridine nucleotide. The enzyme was completely inhibited on addn. of *N*-ethylmaleimide or *p*-chloromercuribenzoate, and did not require a bivalent cation for activity. Attempts to demonstrate the reversibility of side chain redn. of lanosterol during both anaerobic and aerobic incubations were not successful. Triparanol and two other inhibitors of cholesterol biosynthesis blocked the redn. of both lanosterol and desmosterol *in vitro*. Unlabeled lanosterol or desmosterol added to the incubation medium caused a comparable inhibition of redn. of C^{14}-lanosterol. It is possible that a single enzyme is responsible for the redn. of both sterol substrates. **V. The time course and pathway of the later stages of cholesterol biosynthesis in the livers of intact rats.** DeWitt S. Goodman, Joel Avigan, and Daniel Steinberg. *Ibid.* 1287-93. Studies have been conducted of the time course of the distribution of radioactivity in rat liver nonsaponifiables at several short intervals after the intravenous injection of 2-C^{14}-DL-mevalonic acid. Recently developed thin-layer chromatographic techniques were employed that permit sepn. of many of the sterol intermediates in cholesterol biosynthesis. Both normal and triparanol-fed rats were studied, and biochem. techniques were used to aid in the identification of some of the intermediate compds. The appearance of radioactivity in liver sterol was extremely rapid. After 2 min. 7% of the injected radioactivity was present in liver nonsaponifiables, and 43% of this was contained in sterols; 57% of the nonsaponifiable radioactivity was present as squalene. After 30 min., 11% of the injected radioactivity was present in the nonsaponifiables, and 89% of this was contained in sterols. Within the sterol fraction, radioactivity was found primarily in lanosterol, an intermediate zone, Δ^7 ($+\Delta^8$)-cholestenol, and cholesterol. The relative amt. of radioactivity in the first three of these decreased progressively from the max. found at 2 min., which is consistent with the conclusion that these components lie on the major biosynthetic pathway to cholesterol. After 2 min., 53% of the sterol radioactivity was in lanosterol and only 19% in cholesterol; by 30 min., 76% of the sterol radioactivity was in cholesterol. The evidence presented suggests that the radioactivity in the intermediate zone from normal rats was contained in a C_{28} sterol mixt. contg. compds. with both satd. and unsatd. side chains. The results also indicate that in normal rats no significant radioactivity was contained in $\Delta^{7,24}$-cholestadienol or in zymosterol, whereas major amts. of radioactivity were present in one or both of these compds. in triparanol-treated rats. Only traces of radioactivity were found in 24,25-dihydrolanosterol and in desmosterol throughout the time period studied. It is probable that neither of these compds. lies on the major normal pathway of cholesterol biosynthesis. Redn. of the side chain probably occurs mainly at some intermediate stage in the sequence of reactions that modify the configuration of the sterol nucleus. Side chain redn. does not occur exclusively at any one point, however, but does occur to different degrees at several or perhaps at all points in the normal pathway from lanosterol to cholesterol. CA

Fate of injected plasmalogen in rabbits. A. F. Robertson and W. E. M. Lands (Univ. of Michigan, Ann Arbor). *J.*

L30 ANSWER 1 OF 3 HCA COPYRIGHT 2006 ACS on STN

AN 62:91232 HCA Full-text

OREF 62:16336c-f

TI Pharmacological 3β-(aminomethoxy)-5-androsten-17-ones

PA Upjohn Co.

SO 8 pp.

DT Patent

LA Unavailable

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	**NL 6404875**		19641102	NL 1964-4875	19640501 <--

see also **US 3172894**

PRAI US 19630501
GI For diagram(s), see printed CA Issue.
AB I, in which R is NH2, NEt2, or NMeCH2CH2OH, are prepared by halogenating 3β-(carboxymethoxy)-5-androsten-17-one (II), and then treating the formed 3β-(haloformylmethoxy)-5-androsten-17-one with NH3, NHEt2, or methylaminoethanol. Thus, 2 g. II is dissolved in 25 ml. tetrahydrofuran (THF) containing 3 drops pyridine and 5 ml. oxalyl chloride. The mixture is stirred 30 min. at 0°, then 30 min. at room temperature, the solution concentrated in vacuo <25°, and 25 ml. anhydrous C6H6 added. The solution is evaporated to dryness in vacuo, the obtained chloroformylmethoxy compound dissolved in 50 ml. THF, and 5 ml. NHEt2 added at 0°. The mixture is stirred 2 hrs. at room temperature, evaporated in vacuo, the residue dissolved in AcOEt, the solution washed successively with H2O, dilute acid, dilute base and H2O, and dried over anhydrous sulfate. The organic solution is evaporated and recrystd. from a mixture of Skellysolve B hexanes and ether (3:2), and then recrystd. (ether) to yield 1.45 g. 3β-(diethylcarbamoylmethoxy)-5-androstan-17-one (I, R = NEt2) (Ia), m. 110-10.5°, [α]D 5° (CHCl3). Ia is useful as sedative and diuretic.

L30 ANSWER 2 OF 3 HCA COPYRIGHT 2006 ACS on STN
AN 62:91231 HCA Full-text
OREF 62:16336a-c
TI Steroid[3,2-b]pyridines
IN Shimizu, Masao; Ota, Motokichi; Ueno, Katsujiro; Takegoshi, Toshio
PA Daiichi Seiyaku Co., Ltd.
SO 5 pp.
DT Patent
LA Unavailable
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	**JP 40003917**	B4	19650302	JP	19620504 <--

PRAI JP 19620504
GI For diagram(s), see printed CA Issue.
AB A mixture of 4 g. 2-hydroxymethylene-17β-hydroxyandrostan-3-one, 2 g. cyanoacetamide, 400 ml. EtOH, and 9.0 ml. NEt3 is refluxed for 10 hrs., concentrated in vacuo, to the residue is added H2O, and precipitated mass is recrystd. from MeOH (or AcOEt) to give I (R = CN, R' = O, R'' = H), m. >300°. Similarly are prepared the following I (R, R', R'', and m.p. given): CN, O, Me, >300°; CN, S, H, >300°; CN, S, Me, >300°. Also are prepared the following II (R, R', R'', and m.p. given): CONH2, NH2, Me, 250-2.5° (AcOEt); CONH2, NH2, H (having a double bond between 4-5), 274-6.5° (decomposition)(MeOH); CONH2, NH2, Me (having a double bond between 4-5), 196-7°/276-80° (double m.p.) (AcOEt); CO2Et, NH2, H, 140° (decomposition)(MeOH); CO2Et, NH2, Me (having a double bond between 4-5), 183-7° (MeOH).

L30 ANSWER 3 OF 3 HCA COPYRIGHT 2006 ACS on STN
AN 59:61307 HCA Full-text
OREF 59:11197c
TI 3β-(Dialkylaminoalkoxy)-5-androstan-17-ones in hypocholesterolemics
IN Kagan, Fred
PA Upjohn Co.
SO 9 pp.
DT Patent
LA Unavailable

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	**FR M1721**		19630408	FR	<--

PRAI US 19610109
OS MARPAT 59:61307
AB 3β-(Dialkylaminoalkoxy)-5-androsten-17-ones, 3β- (dialkylaminoalkoxy)-5α-androstan-17-ones, and their N-oxides are used in hypocholesterolemic compns. The dosage of the active ingredient is approx. 5-150 mg. 1-4 times a day.

L31 ANSWER 4 OF 5 HCA COPYRIGHT 2006 ACS on STN
AN 58:68617 HCA

Full Text

OREF 58:11781f-h,11782a-b
TI Cholesterol biosynthesis. V. The time course and pathway of the later stages of cholesterol biosynthesis in the livers of intact rats
AU Goodman, DeWitt S.; Avigan, Joel; Steinberg, Daniel
CS U.S. Public Health Serv., Bethesda, MD
SO Journal of Biological Chemistry (1963), 238, 1287-93
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA Unavailable
AB Studies have been conducted of the time course of the distribution of radioactivity in rat liver nonsaponifiables at several short intervals after the intravenous injection of 2-C14-DL-mevalonic acid. Recently developed thin-layer chromatographic techniques were employed that permit separation of many of the sterol intermediates in cholesterol biosynthesis. Both normal and triparanol-fed rats were studied, and biochem. techniques were used to aid in the identification of some of the intermediate compds. The appearance of radio-activity in liver sterol was extremely rapid. After 2 min. 7% of the injected radioactivity was present in liver nonsaponifiables, and 43% of this was contained in sterols; 57% of the nonsaponifiable radioactivity was present as squalene. After 30 min., 11% of the injected radioactivity was present in the nonsaponifiables, and 89% of this was contained in sterols. Within the sterol fraction, radioactivity was found primarily in lanosterol, an intermediate zone, $\Delta 7$ (+$\Delta 8$)-cholestenol, and cholesterol. The relative amount of radioactivity in the first three of these decreased progressively from the maximum found at 2 min., which is consistent with the conclusion that these components lie on the major biosynthetic pathway to cholesterol. After 2 min., 53% of the sterol radioactivity was in lanosterol and only 19% in cholesterol; by 30 min., 76% of the sterol radioactivity was in cholesterol. The evidence presented suggests that the radioactivity in the intermediate zone from normal rats was contained in a C28 sterol mixture containing compds. with both saturated and unsatd. side chains. The results also indicate that in normal rats no significant radioactivity was contained in $\Delta 7$,24-cholestadienol or in zymosterol, whereas major amts. of radioactivity were present in one or both of these compds. in triparanol-treated rats. Only traces of radioactivity were found in 24,25-dihydrolanosterol and in desmosterol throughout the time period studied. It is probable that neither of these compds. lies on the major normal pathway of cholesterol biosynthesis. Reduction of the side chain probably occurs mainly at some intermediate stage in the sequence of reactions that modify the configuration of the sterol nucleus. Side chain reduction does not occur exclusively at any one point, however, but does occur to different degrees at several or perhaps at all points in the normal pathway from lanosterol to cholesterol.

L31 ANSWER 5 OF 5 HCA COPYRIGHT 2006 ACS on STN
AN 58:68616 HCA

Full Text

OREF 58:11781e-f
TI Cholesterol biosynthesis. IV. Reduction of lanosterol to 24,25-dihydrolanosterol by rat liver homogenates
AU Avigan, Joel; Goodman, DeWitt S.; Steinberg, Daniel
CS U.S. Public Health Serv., Bethesda, MD
SO Journal of Biological Chemistry (1963), 238, 1283-6
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA Unavailable
AB cf. CA 56, 10753i. The anaerobic reduction of labeled lanosterol, biosynthetically prepared from 2-C14-mevalonic acid, to 24,25- dihydrolanosterol has been demonstrated with rat liver homogenates. Enzymic activity was associated with cell particles, mostly with microsomes, and required reduced triphosphopyridine nucleotide. The enzyme was completely inhibited on addition of N-ethylmaleimide or p- chloromercuribenzoate, and did not require a bivalent cation for activity. Attempts to demonstrate the reversibility of side chain reduction of lanosterol during both anaerobic and aerobic incubations were not successful. Triparanol and two other inhibitors of cholesterol biosynthesis blocked the reduction of both lanosterol and desmosterol in vitro. Unlabeled lanosterol or desmosterol added to the incubation medium caused a comparable inhibition of reduction of C14-lanosterol. It is possible that a single enzyme is responsible for the reduction of both sterol substrates.

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 238, No. 4, April 1963
Printed in U.S.A.

Studies of Cholesterol Biosynthesis

V. THE TIME COURSE AND PATHWAY OF THE LATER STAGES OF CHOLESTEROL BIOSYNTHESIS IN THE LIVERS OF INTACT RATS

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(Received for publication, September 7, 1962)

Recent studies on cholesterol biosynthesis have defined the reaction sequence between mevalonic acid and squalene (1–3). Moreover, it is well established that squalene is cyclized to lanosterol, which is then converted to cholesterol (4–8). This conversion requires the removal of three methyl groups (at positions 4,4′, and 14) from lanosterol, a shift of the nuclear double bond from position 8,9 to 5,6, and reduction of the side chain double bond at position 24,25. A large number of sterol intermediates between lanosterol and cholesterol have been isolated from biological sources or shown to be convertible to cholesterol by tissue preparations, or both (9–20). There is still uncertainty, however, about the normal sequence of reactions in this later part of the biosynthetic pathway, particularly with regard to the point at which the side chain double bond is reduced. Thus, it has been shown that rat liver homogenates under anaerobic conditions can reduce the side chain of both lanosterol (21, 22) and desmosterol (24-dehydrocholesterol) (20). Treatment with triparanol and with certain other inhibitors leads to accumulation of desmosterol in animal tissues (19), establishing that all of the modifications of nuclear structure can occur before reduction of the side chain. On the other hand, it has also been shown that 24,25-dihydrolanosterol can be converted to cholesterol by liver homogenates from both normal and triparanol-treated rats (10, 21, 22). Since reduction of the side chain double bond is probably irreversible (21), this indicates that all of the nuclear modifications can also occur after reduction of the side chain. It is clear, therefore, that alternate pathways exist between lanosterol and cholesterol, and that the demonstration of a given enzymatic reaction does not establish it as necessarily being on the major pathway.

In the present study, information about the normal pathway of cholesterol biosynthesis was sought by following the time course of the distribution of radioactivity in liver sterols at short time intervals after the intravenous injection of 2-C[14]-mevalonic acid to normal rats. Recently developed thin layer chromatographic techniques (23), which permit separation of many of the sterols proposed as intermediates in cholesterol biosynthesis, were employed. Both normal and triparanol-fed rats were studied, and biochemical techniques were used to aid in the identification of some of the intermediate compounds.

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EXPERIMENTAL PROCEDURE

The animals employed were male Sprague-Dawley rats weighing 150 to 200 g; they were fed Purina laboratory chow *ad libitum*. Some rats were fed 0.1% triparanol, added to the usual diet for several weeks before the study.

2-C[14]-DL-mevalonic acid was purchased from the Volk Radio-Chemical Company as its dibenzylethylenediamine salt, and was converted to the potassium salt by addition of a slight excess of KOH, extraction of the diamine with light petroleum ether, and neutralization. Thirty to 50 μc of C[14]-mevalonate, contained in 1 to 1.5 ml of 0.85% NaCl solution, were injected into the tail veins of the rats. After specified short time intervals the animals were decapitated and the livers were excised and extracted in a small blender with 25 volumes (volume per weight) of acetone-ethanol (1:1, volume for volume). The time interval between decapitation and blending the liver was not more than 30 to 40 seconds. The subsequently tabulated time intervals are those between injection of radioactive label and decapitation. The total lipid extract from the liver was saponified for 1 hour at 60° under nitrogen with 2% KOH in 50% ethanol, and the neutral lipids present after saponification (subsequently called "nonsaponifiables") were then extracted with light petroleum ether. The liver nonsaponifiable lipids contained from 5 to 11% of the injected radioactivity, which represented 10 to 22% of the active isomer of mevalonic acid.

Thin layer silicic acid chromatography of the nonsaponifiable fraction was performed as described elsewhere (23). The plates were prestained with rhodamine 6G to provide easy visibility of the various components. Samples were usually applied as long bands containing up to 10 mg of nonsaponifiable material per plate. A carrier mixture of lanosterol-dihydrolanosterol (1 mg) was added to the liver nonsaponifiables, and the sample was chromatographed on 20-cm-long plates in benzene-ethyl acetate (5:1, volume for volume). The developed plates showed two bands fluorescent under ultraviolet light: a thin band of lanosterol-dihydrolanosterol with an R_F of about 0.7, and a wider band of cholesterol with an R_F of about 0.5. The cholesterol band also contained all of the other 27 carbon sterols tested (desmosterol, Δ^7-cholestenol, zymosterol, and 7-dehydrocholesterol) and will be referred to as the 27 carbon sterol zone. The two fluorescent zones were usually separated by approximately 1.5 cm; two or three thin bands of ultraviolet light-absorbing (dark) material were visible between them. The strongest of

these bands was often contiguous with the upper border of the 27 carbon sterol zone. The identity of these ultraviolet light-absorbing compounds is not known. For the preparation of biosynthetic lanosterol of high specific radioactivity, thin layer chromatography was performed without adding carrier lanosterol. In these instances, reference spots of lanosterol-dihydrolanosterol were run on the same plates.

After chromatography, the plates were divided into four zones: Zone 1, comprising all of the area less polar than (with R_F greater than) lanosterol; Zone 2, the 30 carbon sterols (lanosterol and dihydrolanosterol); Zone 3, the area between Zone 2 and the 27 carbon sterols; Zone 4, the 27 carbon sterol band. These zones were separately scraped onto filter paper and eluted with $CHCl_3$. Aliquots of the eluates were assayed for radioactivity, and the remainder of the samples was used for further studies. On several occasions the material on the remainder of the plate (from the 27 carbon sterol zone down to the origin) was eluted; the radioactivity recovered from this portion of the plate never constituted more than 2% of the total radioactivity recovered.

In several instances the material eluted from Zones 2 and 3 was separately rechromatographed. In every case more than 90% of the recovered radioactivity was again found in the same zone. This showed that the radioactivity found in these zones was not due to inadequate resolution of the chromatograms. This procedure was also used for preparative purposes, in order to obtain pure fractions representative of Zones 2 and 3.

Further separation of the 27 carbon sterol zone into Δ^5-stenols and Δ^7 ($+\Delta^8$)-stenols was effected by addition of desmosterol and Δ^7-cholestenol as carriers, and by chromatography on 40-cm-long plates for 24 to 36 hours in an atmosphere of CO_2 with benzene-ethyl acetate, 20:1. This resulted in resolution of two completely separated fluorescent bands: an upper one containing cholesterol and desmosterol, and a lower one containing Δ^7-cholestenol. Since compounds that differ only in the presence or absence of the side chain (Δ^{24}) double bond do not separate in this system (cholesterol and desmosterol, lanosterol and dihydrolanosterol) it was assumed that $\Delta^{7,24}$-cholestadienol, if present, would migrate together with Δ^7-cholestenol, and support for this assumption has been obtained from the results in triparanol-fed rats reported below. It has also been assumed, on the basis of known column chromatographic behavior, that Δ^8-stenols would migrate together with Δ^7-stenols in this system. When chromatographed on silicic acid columns, both zymosterol and zymostenol, as well as Δ^7-cholestenol, migrate behind cholesterol and desmosterol (14, 15). In the thin layer chromatographic system used here, zymosterol migrates with Δ^7-cholestenol. Further proof for these assumptions must await studies with pure compounds of known structure.

In the studies which follow, no attempt was made to isolate separately $\Delta^{5,7}$-cholestadienol (7-dehydrocholesterol), which migrates between cholesterol and Δ^7-cholestenol in the above system. The more recently found method (23) for separation, by chromatography on $AgNO_3$-impregnated plates, of 7-dehydrocholesterol from the other C_{27} sterols was not available at the time of this study.

Separation of sterols differing only in the presence or absence of the side chain double bond was achieved by acetylation, followed by chromatography of the steryl acetates on 40 cm-long plates for 7 to 8 hours in hexane-benzene, 5:1 (23).

The total recovery of radioactivity after chromatography was usually 85 to 90% of the amount applied to the plate. On analysis of compounds differing only in the presence or absence of the side chain double bond, the recovery after acetylation and chromatography of the acetylated products was usually 65 to 70%. Simultaneous chromatography or acetylation and chromatography, of various mixtures of pure labeled compounds (including lanosterol, dihydrolanosterol, cholesterol, and desmosterol), resulted in identical recoveries for each compound. This indicated that losses during the analytical procedures probably affected all components equally. In the results which follow, the distribution of radioactivity among sterol components is calculated from the radioactivity actually recovered after the chromatographic analyses.

For studies *in vitro* of the conversion of intermediate compounds to cholesterol, rat liver homogenates were made, as described elsewhere (3, 24), in 0.1 M potassium phosphate buffer, pH 7.4, containing 4 mM $MgCl_2$ and 30 mM nicotinamide, by the method of Bucher and McGarrahan (25). The homogenates were centrifuged at 10,000 $\times$ g for 25 minutes, and the 10,000 $\times$ g supernatant solution, containing microsomes and soluble proteins, was used for the incubations. To 4 ml of this homogenate fraction were added 5 μmoles of TPN and 10 μmoles of glucose 6-phosphate, and then the substrate sterol in 100 μl of acetone solution. Incubations were carried out for 2 hours under oxygen at 37°, and were terminated by the addition of an equal volume of ethanol and 0.1 volume of 40% KOH. After addition of carrier lanosterol-dihydrolanosterol, the mixtures were saponified under nitrogen and the nonsaponifiable fractions were analyzed by thin layer chromatography.

In some experiments $C^{14}O_2$ was collected in a well containing 0.3 ml of 10% KOH solution. At the end of the incubation, 0.25 ml of 6 N H_2SO_4 was injected into the medium, and the flasks were shaken for 30 minutes at room temperature. The KOH was then quantitatively removed and diluted to 2.5 ml with water. Two milliliters of this solution were added to 10 ml of the scintillation mixture described by Bray (26), and the samples were assayed in a Packard liquid scintillation spectrometer with an efficiency of about 40%. Corrections for quenching were made by counting the samples after the addition of internal standard. All other radioassays were carried out in 0.5% diphenyloxazole in toluene with a counting efficiency of 50 to 55%. The two counting systems were compared by assay of the same amount of C^{14} absolute standard in each. The amount of $C^{14}O_2$ recovered was related to the amount of radioactive precursor converted to 27 carbon sterols during the incubation, as determined by radioassay of the total nonsaponifiables, followed by thin layer chromatography and analysis of the distribution of radioactivity as usual.

Reference sterols were obtained from the following sources. Cholesterol was purchased from Nutritional Biochemicals Corporation and was purified via its dibromide (27), followed by several crystallizations from ethanol. Desmosterol and zymosterol were kindly provided by Dr. D. R. Idler. The desmosterol was found to be pure on gas-liquid chromatography, but the zymosterol separated into two component peaks of comparable size. On thin layer chromatography, however, both as the free sterol and after acetylation, this zymosterol preparation migrated as a single component that absorbed ultraviolet light, presumably owing to the presence of impurities or degradation products. In later studies, a reference sample of pure zymosterol, generously provided by Dr. Ivan D. Frantz, Jr., was used. This material migrated as a single fluorescent band having

the same R_F as the preparation used in the earlier studies Δ^7-Cholestenol and 24,25-H^3-dihydrolanosterol were kindly provided by Dr. E. Mosettig, and had been prepared in his laboratory by M. J. Thompson, R. T. Brown, and J. A. Steele; both compounds were pure on analysis by both thin layer chromatography and gas-liquid chromatography. Methostenol (4α-methyl-Δ^7-cholestenol) was obtained from Dr. W. W. Wells. The authors wish to acknowledge with thanks the gifts of all these compounds. The lanosterol-dihydrolanosterol mixture was obtained from the Aldrich Chemical Company. Pure lanosterol and pure dihydrolanosterol were prepared by thin layer chromatography of the mixed steryl acetates.

RESULTS

Distribution of radioactivity in rat liver nonsaponifiables at various intervals after the injection of C^{14}-mevalonate is shown in Table I. At the shortest time intervals studied (2 to 3 minutes), thin layer chromatography Zone 1 contained more than 50%, and Zone 2 more than 20%, of the radioactivity in normal rats. Only 7 to 14% of the nonsaponifiable radioactivity was present in Zone 4 (27 carbon sterols). With increasing time intervals, the amount of radioactivity in Zones 1 and 2 steadily declined, whereas that in Zone 4 markedly increased. After 30 minutes, 74% of the radioactivity was present in the 27 carbon sterol zone.

The distribution of radioactivity in triparanol-fed rats differed somewhat from that in normal rats (Table I). In the former, a higher proportion of radioactivity was present in Zone 2 after 3 and after 10 minutes. Only 2% of the total radioactivity was present in the 27 carbon sterol zone after 3 minutes. In addition, there was a distinct fluorescent band corresponding to lanosterol in location, on the plates after chromatography of nonsaponifiables without addition of carrier lanosterol-dihydrolanosterol. An equivalent band was not observed with preparations from normal rats. These results suggest that treatment with triparanol reduced the rate of conversion of lanosterol to 27 carbon sterols.

Zone 1 (Squalene)—The radioactivity found in Zone 1 was located close to the solvent front, distinctly separate from lanosterol. It was assumed to represent squalene, since this is the only known nonsaponifiable precursor of cholesterol much less polar than lanosterol. This identification was confirmed by chromatography of aliquots of several samples of the liver nonsaponifiables on columns of alumina (Woelm, Grade II) (28). Squalene was eluted with light petroleum ether, followed by elution of all the sterols with acetone-ether (1:1, volume for volume). The percentage of radioactivity found in the petroleum ether fractions was almost identical with that in the corresponding Zone 1. Schneider, Clayton, and Bloch have identified the radioactivity in such petroleum ether eluates as squalene by formation of the thiourea adduct (7), and squalene purified by such column chromatography has been used as a substrate for study of the enzymatic cyclization of squalene to sterol (28).

Zone 2 (Lanosterol)—The radioactivity in Zone 2 corresponded chromatographically to lanosterol and dihydrolanosterol. Support for the identity of Zone 2 radioactivity with a 30 carbon sterol was obtained by biochemical means. Incubation of liver homogenates with radioactive material from Zone 2, purified by two chromatographies without carrier, resulted in the formation of C^{14}-cholesterol in high yield; in some instances more than 80% of the recovered radioactivity was found in the 27 carbon sterol zone. Quantitative experiments were then conducted to compare the yield of C^{14} in CO_2 with that in the 27 carbon sterol zone. This technique for characterizing lanosterol, by means of the ratio of radioactive cholesterol to CO_2, was first described by Olson, Lindberg, and Bloch (5) and by Gautschi and Bloch (9), with the use of lanosterol derived from C^{14}-acetate. A 30 carbon sterol derived from 2-C^{14}-mevalonate would be expected to contain 6 labeled carbon atoms. 1 of which would be located in the gem-dimethyl groups at carbon 4 (8); the 14-methyl group would not be labeled. In one experiment, the yield of C^{14} in CO_2 was 1833 d.p.m., and the 27 carbon sterol zone contained 9176 d.p.m. The ratio of radioactivity in the 27 carbon sterol zone to that in CO_2 hence was 5.0, which is precisely the theoretical ratio expected for a 30 carbon sterol, since loss of the three methyl groups at positions 4,4′, and 14 would result in loss of 1 of 6 labeled carbons as CO_2. In another experiment, the liver nonsaponifiable fraction was obtained from a rat given an injection of 2-C^{14}-acetate 5 minutes before death, and the Zone 2 compounds were purified by two chromatographies. Incubation of this material with liver homogenate resulted in the appearance of 1432 d.p.m. of C^{14} in the 27 carbon sterol zone, and of 308 d.p.m. of $C^{14}O_2$ (ratio 4.36). Lanosterol derived biosynthetically from 2-C^{14}-acetate should contain 18 labeled carbon atoms, including label in all three "extra" methyl groups. The observed ratio of 4.36 hence compares favorably with the expected ratio of 5.

Acetylation and thin layer chromatography of Zone 2, together with carrier lanosterol-dihydrolanosterol, revealed that the radioactivity of Zone 2 was present in all samples almost entirely (95% or more) as lanosterol, with only traces of radioactivity associated with dihydrolanosterol. To check the extent to which the relatively small amount of radioactivity recovered in the dihydrolanosterol band might reflect contamination from the much larger amount of radioactivity in the lanosterol band, the dihydrolanosteryl acetate in one experiment (Rat 2) was eluted and rechromatographed with added carrier lanosteryl acetate. Fifty-four per cent of the recovered radioactivity was found in the dihydrolanosterol zone. After the initial chromatography of the steryl acetates, the dihydrolanosteryl acetate zone had contained 3.9% of the recovered radioactivity and the lanosteryl acetate zone 96.1%. Evidently the radioactivity definitely attributable to dihydrolanosterol was only about 2% of the total in the 30 carbon sterol zone.

Further evidence that the Zone 2 radioactivity resided almost

TABLE I

Percentage distribution of radioactivity in liver nonsaponifiables of normal and triparanol-fed rats at short time intervals after intravenous injection of 2-C^{14}-mevalonate

Thin layer chromatography zone	Identity of zone	Per cent of total radioactivity in:						
		Normal rats					Triparanol-treated rats	
		1 (2 min)	2 (10 min)	3 (30 min)	4 (2 min)	5 (3 min)	T1 (3 min)	T2 (10 min)
1	Squalene	57	30	11	69	55	43	13
2	C_{30} sterols	23	17	7	20	22	51	46
3	Intermediate	6	9	8	4	9	4	19
4	C_{27} sterols	14	44	74	7	14	2	16

entirely in lanosterol was obtained by gas-liquid chromatography, with monitoring of the effluent stream for radioactivity. This was kindly performed by Dr. Arthur Karmen (29). In every sample a distinct peak of radioactivity emerged with the retention time of that of lanosterol. No definite radioactive peak corresponding to dihydrolanosterol was observed, and, in view of the precision of the measurement, this indicated that the dihydro compound contained less than 5% of the radioactivity present in lanosterol.

Separation of C_{27} Δ^5-Stenols from C_{27} Δ^7 (and Δ^8)-Stenols—Carrier desmosterol (0.5 mg) and Δ^7-cholestenol (1 mg) were added to about 1 mg of sterol from Zone 4, and chromatography was performed as described above. Table II shows that after 2 to 3 minutes almost 50% of the C_{27} sterol radioactivity was present in the Δ^7-band, whereas by 30 minutes this percentage had decreased to below 10%. This finding is consistent with a role of Δ^7 compounds, Δ^8 compounds, or both as intermediates in the synthesis of cholesterol. A second chromatography was performed to determine the consistency of chromatographic behavior of the eluted Δ^7 zone. Carrier desmosterol (1 mg) was added, and the chromatography was repeated. Of the recovered radioactivity, 93 to 98% was found in the Δ^7 zone.

C_{27} Δ^5-Stenols: Separation of Cholesterol and Desmosterol—To reduce possible contamination with other fractions, the material eluted from the C_{27} Δ^5 zones was first rechromatographed with carrier Δ^7-cholestenol. The purified Δ^5 zone material was then acetylated, and the esters were chromatographed as described in "Experimental Procedure." The results are shown in Table III. Only very small fractions of the total radioactivity were

TABLE II

Radioactivity in $C_{27}\Delta^7$ ($+\Delta^8$) zone expressed as percentage of total C_{27} sterol radioactivity

Rat	Time	Radioactivity in $C_{27}\Delta^7$ zone (percentage of C^{14} in C_{27} sterols)
	min	
1	2	42
2	10	18
3	30	7.4
5	3	49
T1	3	53
T2	10	28

TABLE III

Distribution of radioactivity between cholesterol and desmosterol in C_{27} Δ^5 zone

Rat	Time	$C_{27}\Delta^5$ radioactivity recovered in desmosterol zone initially	"Desmosterol" radioactivity recovered in desmosterol zone on repeated chromatography	Net desmosterol: per cent of total C^{14} in C_{27} Δ^5 zone
	min	%	%	
1	2	5.3	77	4.1
2	10	5.6	84	4.7
3	30	1.6	36	0.6
5	3	2.0	40	0.8
T1	3	>97		>97

recovered in the desmosterol zone in normal rats, whereas almost all of the label was recovered in desmosterol in the case of the triparanol-treated animal (T1). Because the amount of radioactivity in desmosterol in the normal rats was so small relative to that in cholesterol, it was important to check for overlapping of zones. The desmosteryl acetate was eluted and, after addition of carrier cholesteryl acetate, rechromatographed. As shown in Table III, 20 to 60% of the radioactivity found in the desmosterol zone after the first chromatography was dissociated from it by the second chromatography. The corrected values for the percentage of true desmosterol radioactivity in the C_{27} Δ^5 zones are shown in the last column of Table III. In all samples from normal rats, desmosterol accounted for less than 5% of the total radioactivity in the C_{27} Δ^5 zone.

C_{27} Δ^7 ($+\Delta^8$)-Stenols: Side Chain Saturation—The C_{27} Δ^7-stenol zones from the normal and the triparanol-treated rats were eluted, acetylated, and chromatographed on long plates by the method used to separate sterols differing only in the presence or absence of the side chain double bond (desmosterol from cholesterol, lanosterol from dihydrolanosterol). Carrier Δ^7-cholestenol and, in the absence of available $\Delta^{7,24}$-cholestadienol, zymosterol were added before acetylation. The developed plates showed an upper fluorescent band corresponding to Δ^7-cholestenyl acetate and a lower, immediately adjacent, zymosteryl acetate band. In the case of normal Rats 1, 2, and 3, 90% to 93% of the radioactivity was recovered in the Δ^7-cholestenyl acetate band; in the case of triparanol-treated Rats T1 and T2, less than 7% of the label was recovered in the Δ^7-cholestenyl acetate zone. The acetylated Δ^7-stenol zone material from normal Rat 5 and from triparanol-treated Rat T1 were simultaneously chromatographed on one plate. The upper Δ^7-cholestenyl acetate zone contained 89% of the radioactivity of the sample from the normal rat but none of that in the sample from the triparanol-treated rat. The chromatogram in the latter case showed a faint band of fluorescence extending below the zymosterol band. This, when eluted and counted separately, was found to contain more than half of the radioactivity recovered from the sample. Thus, in triparanol-treated rats the major labeled component in the Δ^7 ($+\Delta^8$)-stenol zone, when chromatographed as the acetate ester, moved more slowly than Δ^7-cholestenyl acetate, which is consistent with the presence of a side chain double bond. This conclusion is also consistent with the known effect of triparanol of inhibiting reduction of the sterol side chain (19, 20) and with the tentative identification by Frantz and Mobberley of $\Delta^{7,24}$-cholestadienol as a highly labeled intermediate during sterol biosynthesis in liver slices from triparanol-treated rats (30). In contrast, in normal rats most of the radioactivity, after acetylation of the Δ^7-stenol zone material, chromatographed together with Δ^7-cholestenyl acetate. This suggests that almost all of the labeled C_{27} Δ^7 ($+$ Δ^8)-stenol from normal rats possessed a saturated side chain.

The presence or absence of the side chain double bond was further checked by a biological approach, based on the ability of triparanol to inhibit reduction of the sterol side chain. Incubations were conducted with various precursor sterols as substrates, with the use of liver homogenates from rats fed 0.1% triparanol for the preceding 2 weeks. The precursor sterols either were of biosynthetic origin from normal and triparanol-treated rats or were synthesized chemically. It was expected that substrates with saturated side chains would yield only cholesterol in the C_{27} Δ^5 zone, whereas substrates with unsatu-

rated side chains would yield mainly desmosterol. Because inhibition of side chain reduction during the incubations was incomplete, the results shown in Table IV had to be related to those obtained with reference compounds of known structure. As a reference compound with a saturated side chain, 24,25 H^3-dihydrolanosterol was used. C^{14}-lanosterol, purified from chromatography Zone 2 from Rat T1, was used as reference for compounds with unsaturated side chains. As expected, when dihydrolanosterol was used as substrate, all (98%) of the C_{27} Δ^5 radioactivity was found in cholesterol. In contrast, when lanosterol was used as substrate only 49% of the C_{27} Δ^5 radioactivity was found in cholesterol, the remaining 51% being in desmosterol. Although there was only partial inhibition of side chain reduction during the incubations, the difference is striking. When the eluted C_{27} Δ^7 zone from normal Rat 5 was used as substrate, virtually all (98%) of the Δ^5 zone radioactivity was found in cholesterol. With the C_{27} Δ^7 zone from triparanol-treated Rat T1 as substrate, however, only 51% of the C_{27} Δ^5 radioactivity was in cholesterol and 49% was in desmosterol. Thus the C_{27} Δ^7 zone radioactivity from the normal rat yielded cholesterol almost exclusively, whereas that from the triparanol-fed rat yielded a desmosterol-cholesterol mixture in the same ratio obtained with reference lanosterol. This adds support to the conclusion that the radioactivity of the "Δ^7" zone was in compounds with saturated side chains in normal rats, and in compounds with unsaturated side chains in triparanol-fed rats.

Zone 3—Only preliminary information has been acquired as to the identity of the radioactive substance or substances in Zone 3. Since methostenol migrates in the center of this zone, we thought it likely that the radioactivity of Zone 3 was contained in C_{28} or C_{29} sterols. In addition, gas-liquid chromatography revealed a definite peak of radioactivity with retention time similar to that of methostenol. Because of technical inadequacies of analyzing the gas chromatographic effluent for radioactivity, it could not be determined with confidence whether the radioactivity peak had a retention time identical with that of methostenol. These analyses also suggested the presence of one or more additional smaller peaks of radioactivity in the same zone and were not entirely satisfactory.

Information about the Zone 3 material was sought by biochemical means. Incubation of this material with liver homogenates showed its conversion to cholesterol in good yield. Examples of such incubations are shown in Table IV. Attempts were then made to determine the number of carbon atoms in the Zone 3 sterol or sterols, and to try to define the position of any "extra" methyl groups present. An incubation was carried out with purified Zone 3 material obtained after two chromatographies from the rat injected with 2-C^{14}-acetate. Radioactivity from the latter is incorporated into all of the "extra" methyl groups of the sterol precursors of cholesterol. After the incubation, 2464 d.p.m. of C^{14} were present in 27 carbon sterols, and 148 d.p.m. in CO_2. The ratio of radioactivity in 27 carbon sterols to that in CO_2 was, thus, 16.7. This suggested that the radioactivity in Zone 3 was mainly present in 28 carbon sterols, since the expected ratio for a 28 carbon sterol is 15, and for a 29 carbon sterol, 7.5. No $C^{14}O_2$ was obtained with C^{14}-cholesterol as control substrate.

To determine the homogeneity of this sterol fraction, an experiment was conducted to find whether the radioactivity in Zone 3 would separate into two peaks on thin layer chromatography after acetylation. Zone 3 material prepared by thin layer chromatography from the "nonsaponifiable" fraction of 40 rat livers was used as carrier. After acetylation and chromatography, the lower half of the plate was divided into several narrow zones. Radioactivity was present as a broad single band. The upper three-fourths of this band was combined into a single sample (Zone 3a), as was the lower one-fourth (Zone 3b). These samples were then hydrolyzed to regenerate the free sterol, and incubations were conducted as shown in Table IV. Different results were obtained with the two fractions. Most (89%) of the C_{27} Δ^5-stenol derived from Zone 3a was cholesterol, compared to only 51% from Zone 3b. This suggests that most of Zone 3b sterol contained an unsaturated side chain, whereas most of the Zone 3a had a saturated side chain. The unfractionated Zone 3 gave, on incubation, 79 to 86% of the C_{27} Δ^5 radioactivity in the form of cholesterol (Table IV). This indicated that Zone 3, as a whole, contained a preponderance of compounds with saturated side chains. In contrast, only 57% of the C_{27} Δ^5 radioactivity derived from Zone 3 from the triparanol-treated rat (T1) was found in cholesterol. This showed that in Zone 3, as well as in the other chromatographic zones, the labeled sterols from the triparanol-treated rat were almost entirely unsaturated in the side chain.

TABLE IV

Conversion of intermediate sterols to cholesterol or desmosterol with liver homogenates from triparanol-treated rats

Substrate	Source	Per cent of recovered radioactivity		Per cent of C_{27} Δ^5 radioactivity as cholesterol
		C_{27} Δ^7	C_{27} Δ^5	
H^3-dihydrolanosterol	Chemical labeling	25	26	98
C^{14}-lanosterol	Rat T1	23	65	49
C^{14}-C_{27} Δ^7 zone	Rat T1	24	76	51
	Rat 5	13	87	98
C^{14}-Zone 3, total	Rat T1	34	54	57
	Rat 5	27	42	79
C^{14}-Zone 3, total (repeat experiment)	Rat 5	22	49	86
C^{14}-Zone 3a*	Rat 5	22	35	89
C^{14}-Zone 3b*	Rat 5	30	43	51

* See the text.

DISCUSSION

The results summarized in Table V show the rapidity with which intravenously injected 2-C^{14}-mevalonate appeared in liver sterol. As early as 2 minutes after injection, the percentage of injected radioactivity recovered in liver nonsaponifiables was almost as great, and at 10 minutes it was as great, as that recovered after 30 minutes. Two minutes after injection, 43% of the radioactivity in the nonsaponifiable fraction was present as sterol. By 30 minutes, the radioactivity in the sterols had risen to 89% of the total and only 11% remained in squalene. Within the sterol fraction, radioactivity was found primarily in lanosterol, in the intermediate Zone 3 (C_{28} sterols), in Δ^7-cholestenol, Δ^8-cholestenol, or both, and in cholesterol. The relative amount of radioactivity in lanosterol, Zone 3, and the cholestenols decreased progressively from the maximum found at 2 minutes, which is consistent with the conclusion that these components lie on the major biosynthetic pathway to choles-

TABLE V

Time course of distribution of radioactivity in rat liver nonsaponifiables after 2-C^{14}-DL-mevalonate

	Rat		
	1 (2 min)	2 (10 min)	3 (30 min)
A. Per cent of injected radioactivity in liver nonsaponifiables	7	11	11
B. Per cent of total nonsaponifiable radioactivity:			
Squalene	57	30	11
Sterol	43	70	89
C. Per cent of sterol radioactivity:			
Lanosterol	53	24	8
Dihydrolanosterol	<2.5	0.5	<0.5
Intermediate (Zone 3)	14	13	9
Δ^7 (+Δ^8)-Cholestenol	14	11	6.1
Desmosterol	0.8	2.3	0.5
Cholesterol	19	50	76

terol. Lanosterol, of course, is well established as the first sterol intermediate in the pathway (7, 8), and the present results thus extend the observations of Schneider, Clayton, and Bloch (7). The relatively large fraction of radioactivity present in lanosterol at 2 and 10 minutes suggests that the cyclization of squalene is not a rate-limiting step.

Because the components in Zone 3 were not identified, only tentative conclusions can be drawn about the sterols in this fraction. Zone 3 would be expected to include C_{28} and C_{29} sterols in the chromatographic system used. Several 28 and 29 carbon sterol intermediates have been identified from biological sources. Gautschi and Bloch identified radioactive $\Delta^{8,24}$-4,4′-dimethylcholestadien-3β-ol (14-norlanosterol) from the nonsaponifiables from intestines plus livers of rats injected 10 minutes previously with C^{14}-acetate (9). Kandutsch and Russell identified 4-methyl-Δ^8-cholestenol from preputial gland tumors of mice, and showed that this was convertible to cholesterol by rat liver homogenates (10). Wells and Lorah (11) and Neiderhiser and Wells (12) identified labeled 4-methyl-Δ^7-cholestenol (methostenol) in the nonsaponifiables of the skin and of the combined liver and small intestine of rats given C^{14}-acetate, and showed that this methostenol was converted to cholesterol in high yield. In the present work, comparison of the yield of $C^{14}O_2$ with the yield of cholesterol-C^{14} on incubation of 2-C^{14}-acetate-labeled Zone 3 material with a normal liver homogenate suggested that most of the radioactivity resided in C_{28} sterols. Further work is needed to define this fraction.

The finding that as much as 14% of the total sterol radioactivity at 2 minutes was present in Δ^7 (+ Δ^8)-cholestenol strongly suggests that this compound is on the major pathway. Frantz *et al.* (13, 14) have shown that Δ^7-cholestenol is readily converted to cholesterol by rat liver. The present studies show further that in normal rats almost all of the radioactivity in the Δ^7 (+ Δ^8)-C_{27} fraction was present in compounds with saturated side chains. After acetylation the radioactivity migrated on thin layer chromatography together with reference Δ^7-cholestenol. When the corresponding fraction isolated from the livers of triparanol-treated rats was similarly acetylated and chromatographed, almost all of the radioactivity migrated behind the Δ^7-cholestenol zone, as did reference zymosterol. The results are consistent with the presence of a side chain double bond in the sterol isolated from triparanol-treated rats. Further support for these assignments of side chain structure was obtained by determining whether the compounds gave rise to desmosterol or to cholesterol upon incubation with liver homogenates from triparanol-treated rats (Table IV).

Stokes and Fish have reported that relatively larger amounts of radioactive desmosterol were found in rat liver nonsaponifiables 5, 15, and 120 minutes after the injection of C^{14}-acetate (17). In their study, desmosterol represented 4.9%, 3.8%, and 2.3% of the total sterol (digitonide-precipitable) radioactivity at the three time intervals studied. In the early stages of this study, before the separation of the 27 carbon sterols into Δ^7- and Δ^5-compounds had been developed, we in fact concluded incorrectly that much larger amounts of radioactivity were located in desmosterol (22). Almost all of this radioactivity was later found to be present in Δ^7-stenols.

Since only traces of radioactivity were found in dihydrolanosterol and in desmosterol during the time period studied, it is probable that neither dihydrolanosterol nor desmosterol lies on the major pathway of cholesterol biosynthesis. This conclusion must be tentative in the absence of information about the size and turnover rate of the available pools of each of the intermediate sterols. It is conceivable, for example, that no significant radioactivity would be observed in a component on the main pathway because of a small pool size and very rapid turnover rate. If this were the explanation for the low recovery of radioactivity in dihydrolanosterol, the metabolically active dihydrolanosterol pool in rat liver would have to be at least 20 times smaller than the corresponding lanosterol pool. However, our preliminary gas-liquid chromatographic studies[1] have suggested that the amounts of these two sterols in rat liver are similar. Even stronger evidence exists against the possibility of desmosterol being on the main pathway. This derives from the demonstration that the major amounts of radioactivity found in the C_{27} Δ^7 (+ Δ^8)-fractions were always present almost entirely in compounds with saturated side chains. Since Δ^7-cholestenol thus appears to be on the major pathway as a precursor of cholesterol, desmosterol could only be on the major pathway if reduction of the side chain double bond were reversible. There is no evidence that desaturation of the side chain can occur, and evidence to the contrary exists for the cases of dihydrolanosterol (21) and Δ^8-cholestenol (15).

Although the amounts of radioactivity in dihydrolanosterol and in desmosterol were very small, careful, repeated chromatography showed that they were real. It has been reported elsewhere that rat liver contains enzymes capable of reducing lanosterol to the dihydro compound (21, 22), and also of converting lanosterol to desmosterol with retention of the side chain double bond (18, 19). In addition, the biological studies of the material in intermediate Zone 3 (Table IV) indicated that it was probably a mixture of side chain saturated and side chain unsaturated C_{28} sterols.

On the basis of these results, it is concluded that reduction of the side chain double bond does not occur primarily either at the very beginning or at the very end of the sequence of reactions that modify the configuration of the sterol nucleus. Thus, reduction of lanosterol and reduction of desmosterol are probably not quantitatively important steps in the normal pathway of

[1] Unpublished experiments.

cholesterol biosynthesis in rat liver. Furthermore, the failure to find any significant amount of radioactivity in side chain unsaturated compounds in the C_{27} Δ^7-stenol fraction, and the biological evidence cited above with regard to the Zone 3 material, suggests that side chain reduction at the 28 carbon stage may be quantitatively important. The results show further that reduction of the side chain does not occur exclusively at any one given point, but that it can occur to different degrees at several or perhaps at all points in the normal pathway from lanosterol to cholesterol.

SUMMARY

Studies have been conducted of the time course of the distribution of radioactivity in rat liver nonsaponifiables at several short intervals after the intravenous injection of 2-C^{14}-DL-mevalonic acid. Recently developed thin layer chromatographic techniques were employed that permit separation of many of the sterol intermediates in cholesterol biosynthesis. Both normal and triparanol-fed rats were studied, and biochemical techniques were used to aid in the identification of some of the intermediate compounds.

The appearance of radioactivity in liver sterol was extremely rapid. After 2 minutes, 7% of the injected radioactivity was present in liver nonsaponifiables, and 43% of this was contained in sterols; 57% of the nonsaponifiable radioactivity was present as squalene. After 30 minutes 11% of the injected radioactivity was present in the nonsaponifiables, and 89% of this was contained in sterols. Within the sterol fraction, radioactivity was found primarily in lanosterol, an intermediate zone, Δ^7 (+ Δ^8)-cholestenol, and cholesterol. The relative amount of radioactivity in the first three of these decreased progressively from the maximum found at 2 minutes, which is consistent with the conclusion that these components lie on the major biosynthetic pathway to cholesterol. After 2 minutes, 53% of the sterol radioactivity was in lanosterol and only 19% in cholesterol; by 30 minutes, 76% of the sterol radioactivity was in cholesterol.

The evidence presented suggests that the radioactivity in the intermediate zone from normal rats was contained in a C_{28} sterol mixture containing compounds with both saturated and unsaturated side chains. The results also indicate that in normal rats no significant radioactivity was contained in $\Delta^{7,24}$-cholestadienol or in zymosterol, whereas major amounts of radioactivity were present in one or both of these compounds in triparanol-treated rats.

Only traces of radioactivity were found in 24,25-dihydrolanosterol and in desmosterol throughout the time period studied. It is probable that neither of these compounds lies on the major normal pathway of cholesterol biosynthesis. Reduction of the side chain probably occurs mainly at some intermediate stage in the sequence of reactions that modify the configuration of the sterol nucleus. Side chain reduction does not occur exclusively at any one point, however, but does occur to different degrees at several or perhaps at all points in the normal pathway from lanosterol to cholesterol.

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THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 238, No. 4, April 1963
Printed in U.S.A.

Studies of Cholesterol Biosynthesis

IV. REDUCTION OF LANOSTEROL TO 24,25-DIHYDROLANOSTEROL BY RAT LIVER HOMOGENATES*

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(Received for publication, September 7, 1962)

One of the reactions involved in the biosynthesis of cholesterol is the reduction of a side chain double bond, present in lanosterol at the 24,25-position. Although it is not clear which one of the series of sterol intermediates normally undergoes this reduction, it has been shown that 24,25-dehydrocholesterol (desmosterol) accumulates *in vivo* under the influence of certain inhibitors (1–3), and that in normal animals this sterol is rapidly converted *in vivo* (4, 5) and *in vitro* (6) to cholesterol. No similar information is available on the enzymatic reduction of the side chain of lanosterol, but the presence of 24,25-dihydrolanosterol in skin sterols indicates that lanosterol may also undergo biological hydrogenation of its side chain. In addition, Kandutsch and Russell (7) have observed the formation of 24,25-dihydrolanosterol by mouse preputial tumor tissue and have demonstrated the conversion of that sterol to cholesterol by homogenates of preputial tumor, and to a lesser extent by liver. In the present study, the reduction of lanosterol to 24,25-dihydrolanosterol by fractions of rat liver homogenates was investigated.

EXPERIMENTAL PROCEDURE

Lanosterol labeled with C^{14} was prepared biosynthetically from 2-C^{14}-mevalonic acid. The latter was purchased as its dibenzylethylenediamine salt, and was converted to the potassium salt by alkalinization with KOH, extraction of the amine with light petroleum ether, and neutralization. The C^{14}-mevalonate was injected intravenously into rats (approximately 50 μc per rat), and after 2 to 10 minutes the livers were excised and homogenized in a small blender with 25 volumes of acetone-ethanol (1:1).

The resulting lipid extract was saponified with 2% KOH in 50% ethanol under nitrogen for 1 hour, and the nonsaponifiable lipids were extracted with light petroleum ether and fractionated by thin layer chromatography as described in detail elsewhere (8). In brief, the method consisted of chromatography of the material with benzene-ethyl acetate (5:1) on glass plates, 20 × 20 cm, covered with Silica Gel-G. To make the zones visible under ultraviolet light the dye, rhodamine 6G, was incorporated into the thin layer. This procedure separated the 30 carbon sterols (approximate R_F 0.7) from those containing 27 to 29 carbons (R_F 0.5 for cholesterol) and from squalene (R_F 0.95).

* A preliminary report has been published in *Federation Proc.*, **21**, 300 (1962).

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The lanosterol fraction was eluted and rechromatographed in a concentrated band, and the purified sterol was eluted and used as substrate. It was found that rats fed 0.1% triparanol for several weeks yielded more C^{14}-lanosterol than normal animals (9). The absence of labeled 24,25-dihydrolanosterol was demonstrated by thin layer chromatography as described below. The mass of lanosterol was approximately estimated from the visual intensity of the lanosterol band after the second chromatography. The specific radioactivity calculated from this value and from the total radioactivity was about 5000 c.p.m. per μg. Evidence for purity of the product was furnished by gas-liquid chromatography on a column containing a support coated with SE-30 silicon rubber with monitoring of the effluent stream for radioactivity. On aerobic incubation with rat liver homogenates, the material was converted to cholesterol in high yield. The ratio of radioactivity in C^{14}-cholesterol formed to that in $C^{14}O_2$ released was almost exactly 5.0, as predicted for a 30 carbon sterol originating from 2-C^{14}-mevalonate.

Rat liver homogenates were made, as described in detail elsewhere (10, 11), in 0.1 M potassium phosphate buffer, pH 7.4, containing 4 mM $MgCl_2$ and nicotinamide, by the method of Bucher and McGarrahan (12). The 2,000 × *g* supernatant solution, mitochondria, microsomes, and the "104,000 × *g* supernatant" were prepared from the homogenates as described (10, 11). Particles were washed once and then suspended in a volume of buffer equal to one-fifth the volume of the liver homogenate from which they had been prepared.

Incubations were conducted in 25-ml Erlenmeyer flasks at 37° in a shaking incubator, for 2 hours, under a stream of nitrogen saturated with water. Each flask contained homogenate fractions as indicated, 0.1 M KPO_4 buffer, pH 7.4, 0.03 M nicotinamide, and other additions as indicated; the final volume was usually 4.5 ml. Labeled lanosterol (usually about 5000 c.p.m. in approximately 1 μg) was added in 100 μl of acetone solution to the ice-cold incubation mixture. At the end of the incubation the flask contents were transferred to tubes containing 1 ml of 40% KOH, and the flasks were washed with 5 ml of ethanol. Carrier lanosterol-24,25-dihydrolanosterol mixture (2 mg) was added, and the samples were saponified at 60° for 1 hour under nitrogen. The nonsaponifiable fraction was extracted with light petroleum ether and fractionated by thin layer chromatography as described above. The band containing the 30 carbon sterols was scraped from the plate and eluted with methylene chloride or chloroform. The sterols were then acetylated, and lanosteryl

acetate and 24,25-dihydrolanosteryl acetate were separated by a second thin layer chromatography on 40-cm-long plates in hexane-benzene (5:1, volume for volume) (8). The two zones were separated by a distance of 3 to 4 cm with very little if any cross-contamination. Each compound was separately eluted and radioassayed in a Packard liquid scintillation spectrometer. The percentage of substrate reduced was calculated as the percentage of C^{14}-24,25-dihydrolanosterol in the labeled dihydrolanosterol and lanosterol recovered after the second chromatography. Control experiments with C^{14}-lanosterol and H^3-24,25-dihydrolanosterol, used together or individually, showed that the overall recovery of each sterol carried through the above procedure was about 75%. The recoveries of the two were not significantly different. The specific radioactivity of a mixture of 1 mg of C^{14}-24,25-dihydrolanosteryl acetate isolated from the incubation and 24 mg of carrier 24,25-dihydrolanosteryl acetate did not change significantly after three recrystallizations from methanol (from 14 to 13 c.p.m. per mg). It should be noted that when a mixture of C^{14}-lanosteryl acetate and carrier 24,25-dihydrolanosteryl acetate was similarly recrystallized three times from methanol, the specific radioactivity was reduced from 15 to 7 c.p.m. per mg. The nonlabeled lanosterol-24,25-dihydrolanosterol mixture was obtained by column chromatography of a commercial product. It was free of contaminations detectable by ultraviolet and infrared spectroscopy and gave only the two peaks on gas-liquid chromatography, identified as the two above mentioned sterols by comparison with two samples of the same compounds obtained from Dr. D. R. Idler and Dr. P. D. Klein. The material was chromatographed at 235° in the form of free sterols on a column containing an SE-30 coated phase; 24,25-dihydrolanosterol emerged at 21 minutes and lanosterol at 23.5 minutes. Pure lanosterol was prepared by thin layer chromatography of the acetylated sterol preparation mentioned above. Desmosterol was synthesized and kindly provided by Drs. D. R. Idler and E. Reiner of the Fisheries Research Board of Canada. 24,25-H^3-dihydrolanosterol (approximately 3×10^6 c.p.m. per mg) was prepared in the Section on Steroids, Laboratory of Chemistry, National Institute of Arthritis and Metabolic Diseases. Triparanol (1-[*p*-(β-diethylaminoethoxy]phenyl)-1-(*p*-tolyl)-2-(*p*-chlorophenyl)-ethanol) was obtained from the William S. Merrell Company, and the experimental drugs, U-18,666A (3β-(2-diethylaminoethoxy)androst-5-en-17-one) and U-5755 (hexestrol bis-β-diethylaminoethyl ether hydrochloride), from the Upjohn Company.

RESULTS

Incubation of various subcellular fractions with labeled lanosterol revealed that the enzymatic activity for side chain reduction resided in particles, mostly in microsomes (Table I). The slight activity shown by the soluble fraction alone in Experiment 1 was probably due to the presence of residual microsomes, since a second centrifugation at 104,000 × *g* (Experiment 2) removed this activity almost completely.

Table I shows that the sum of the activities of the three fractions incubated individually was considerably less than that of the unfractionated homogenate. (It should be noted that the particulate fractions incubated separately were used at twice their concentration in the whole homogenate. The values in parentheses for Experiment 1 show the activities of the particulate fractions corrected to the concentrations of the particles in the whole homogenate, on the assumption that activity is linearly related to particle concentration.) Adding 104,000 × *g* supernatant to washed microsomes and mitochondria (Experiment 1) or to washed microsomes (Experiment 2) increased the yield by 101% and 45%, respectively, over that obtained with individual fractions and corrected for the quantity of each in the "combined fractions" (Table I). The stimulation was not observed with heat-denatured soluble fraction. A similar stimulation by a soluble fraction, inactive when used alone, of an enzymatic reduction of a sterol (7-dehydrocholesterol) was reported by Kandutsch (13).

Table II shows the pyridine nucleotide requirement for this reaction. In the absence of added pyridine nucleotide, or with DPNH, no reduction of lanosterol was observed. Maximal yields were obtained with TPNH. Surprisingly, however, considerable activity was observed on addition of TPN. An attempt was made to determine whether the activity observed with TPN was due to reduction of TPN to TPNH during the incubation. An aliquot of the microsome suspension was incubated with TPN for 30 minutes, and the optical density at 340 mμ was compared with that of a control incubation lacking TPN. The optical density relative to that of the control flask was 0.015, indicating reduction of less than 0.5% of the TPN added. Hence significant reduction of TPN was not demonstrated. It should be noted, however, that because of the very large excess of the nucleotide used in these experiments, the reduction of a small fraction of the TPN could be adequate for the reduction of lanosterol.

As shown in Table II, Mg ions were not obligatory for this reaction. The two —SH inhibitors tested, *N*-ethylmaleimide

TABLE I

Anaerobic reduction of C^{14}-lanosterol with fractions from rat liver homogenates

The incubation flasks contained enzyme, buffer, and 2.5 μmoles each of TPN, DPNH, and TPNH in a final volume of 4.5 ml. The amounts of enzyme fractions used were: whole homogenate and 104,000 × *g* supernatant, 4 ml; mitochondria and microsomes, 1.6 ml. In Experiment 1, the "combined fractions" included 104,000 × *g* supernatant, 2.4 ml, and 0.8 ml each of mitochondria and microsomes. In Experiment 2, "combined fractions" referred to 104,000 × *g* supernatant, 2.4 ml, and microsomes, 1.6 ml. The flask with "particles + boiled supernatant" in Experiment 1 contained 0.8 ml each of mitochondria and microsomes and 2.4 ml of the clear supernatant solution obtained from a 10,000 × *g* centrifugation of a portion of 104,000 × *g* supernatant which had been immersed in boiling water for 5 minutes.

Enzyme fraction	C^{14}-lanosterol reduced	
	Experiment 1	Experiment 2
	%	%
Whole homogenate	48	—*
Mitochondria	8 (4)†	—
Microsomes	14 (7)†	18.3
104,000 × *g* supernatant	4	1.3‡
Combined fractions	27	27.8
Particles + boiled supernatant	15	—

* The dash (—) means not studied.

† Values in parentheses denote activities corrected to the concentration of particles in the whole homogenate.

‡ Centrifugated twice at 104,000 × *g* for 60 minutes.

TABLE II

Cofactor requirements and effects of —SH inhibitors on anaerobic reduction of C^{14}-lanosterol by rat liver microsomes

Homogenates were prepared, and microsomes washed, with 0.1 M KPO_4 buffer, pH 7.4, containing no added nicotinamide. In Experiment 2, $MgCl_2$ was also omitted, and the buffer contained, instead, 0.5 mM EDTA. Each flask contained, in a final volume of 4 ml, 1.8 ml of washed microsome suspension, 400 μmoles of KPO_4 buffer, 120 μmoles of nicotinamide, 20 μmoles of $MgCl_2$ (except where indicated), and 5 μmoles of the nucleotides, when added as indicated below.

Addition or omission	C^{14}-Lanosterol reduced	
	Experiment 1	Experiment 2
	%	%
No pyridine nucleotide	0	—*
DPNH	0	0.4
TPN	11.9	27
TPNH	18.3	31
No Mg^{++}; TPNH	—	21
N-Ethylmaleimide (8 μmoles) + TPNH	—	0
p-Chloromercuribenzoate (3 μmoles) + TPNH	—	0

* The dash (—) means not studied.

and p-chloromercuribenzoate, both completely inhibited the reduction of lanosterol.

Several compounds known to block the conversion of desmosterol to cholesterol were found to inhibit the reduction *in vitro* of the lanosterol side chain as shown in Table III. Two incubations were made with each compound; one under nitrogen and the other under oxygen. The anaerobic samples were analyzed as usual, and the percentage of radioactivity present as 24,25-dihydrolanosterol in the 30 carbon sterol zone was determined. Table III shows that all compounds tested inhibited the reduction of lanosterol by 95% or more. In the aerobic samples, the 27 carbon sterol zones were found to contain 75 to 83% of the total radioactivity (Table III), indicating that none of the compounds tested significantly inhibited the aerobic oxidation of lanosterol to 27 carbon sterols. Carrier desmosterol (1 mg) was then added to the 27 carbon sterols isolated from each incubation, and the mixture was acetylated and fractionated by thin layer chromatography into cholesteryl and desmosteryl acetates in the same system used for separation of lanosterol from 24,25-dihydrolanosterol (8). Triparanol, U-18,666A, and U-5755 all significantly inhibited the reduction of the sterol side chain (Table III), but the effects under aerobic conditions were consistently less than under anaerobic conditions.

In other experiments it was found that homogenates of livers from rats fed 0.1% triparanol were incapable of anaerobically reducing lanosterol. Under aerobic conditions, however, the nuclear transformations could take place and in one experiment the same homogenate converted 30% of added C^{14}-lanosterol to desmosterol, and 18% of added H^3-24,25-dihydrolanosterol to cholesterol.

Experiments were conducted to determine whether the reduction of the side chain of lanosterol might be reversible. Anaerobic incubation of C^{14}-24,25-dihydrolanosterol (obtained by enzymatic reduction of C^{14}-lanosterol) with normal liver homogenate in the presence of excess TPN showed no conversion of 24,25-dihydrolanosterol to lanosterol. Furthermore, after an aerobic incubation of liver homogenate with 17,750 c.p.m. of 24,25-H^3-dihydrolanosterol as substrate, 15,700 c.p.m. were recovered in the combined sterol fractions after thin layer chromatography. Cholesterol contained 41%, the C_{30} sterols, 46%, and the intermediate zone, 13% of the radioactivity. No H^3-lanosterol was recovered after carrier nonlabeled lanosterol was added to the material eluted from the zone containing the 30 carbon sterols, acetylation, and repeated chromatography. If significant desaturation of the side chain of 24,25-H^3-dihydrolanosterol had occurred, some H^3 would have been expected to be lost from the total sterol fraction and some H^3-lanosterol formed. Similar results have been reported for the conversion of 24,25-H^3-Δ^8-cholestenol to cholesterol (14).

The capacity of the system *in vitro* to reduce lanosterol was investigated in experiments in which varying amounts of carrier lanosterol were mixed with a constant amount of C^{14}-lanosterol and the resulting mixtures were added in 100 μl of acetone to incubation flasks. In other flasks, similar amounts of unlabeled desmosterol were added to the labeled lanosterol to test for possible substrate competition. In a preliminary experiment, there was no change in the percentage yield of C^{14}-24,25-dihydrolanosterol with the addition of 2, 7, or 30 μg of unlabeled lanosterol or of 10 μg of unlabeled desmosterol. The results of a second experiment, presented in Table IV, showed the relatively

TABLE III

Effects of inhibitors on conversion of lanosterol to 24,25-dihydrolanosterol and to cholesterol

Each flask contained 4 ml of liver homogenate, 2.5 μmoles of TPN, 10 μmoles of glucose-6-P, and 200 μg of each compound tested. Triparanol was added in 50 μl of acetone, and the other inhibitors in 50 μl of water (50 μl of acetone were added to the flasks containing the water-soluble inhibitors). C^{14}-lanosterol in 100 μl of acetone was added last.

Inhibitor added	Anaerobic flasks		Aerobic flasks		
	Dihydrolanosterol in 30 carbon sterols	Inhibition	Total C^{14} as 27 carbon sterols	Cholesterol in 27 carbon sterols	Inhibition
	%	%	%	%	%
None (control)	50	(0)	79	88	(0)
Triparanol	1.3	97	83	51	42
U-18,666A	0.4	99	75	17	80
U-5755	2.7	95	83	42	51

TABLE IV

Effect of unlabeled lanosterol and of desmosterol on anaerobic reduction of C^{14}-lanosterol

Each flask contained 4 ml of liver homogenate, 2.5 μmoles of TPN, 10 μmoles of glucose-6-P, and C^{14}-lanosterol with or without unlabeled lanosterol or desmosterol added in 100 μl of acetone.

Unlabeled lanosterol or desmosterol added	C^{14}-lanosterol converted to C^{14}-24,25-dihydrolanosterol	
	Lanosterol added	Desmosterol added
μg	%	%
0	63	63
50	51	42
150	35	30
500	15	10

high capacity of the enzyme to reduce the substrate. The percentage yield of C^{14}-24,25-dihydrolanosterol from C^{14}-lanosterol with 150 μg of carrier substrate was more than half of that with labeled substrate (approximately 1 μg) alone. Table IV also shows that the addition of desmosterol to labeled lanosterol reduced its conversion to 24,25-dihydrolanosterol at least as effectively as the addition of carrier lanosterol.

DISCUSSION

The results demonstrate that rat liver is able to reduce lanosterol to 24,25-dihydrolanosterol. The enzyme is associated with cell particles, mostly with microsomes, and requires triphosphopyridine nucleotide for activity. The observation that reduction occurred when TPN was added instead of TPNH suggests that catalytic amounts of the nucleotide were reduced in the presence of some endogenous substrate. An attempt to demonstrate directly the reduction of the nucleotide, however, was unsuccessful. It should be noted that fewer than 5 mμmoles of lanosterol were reduced in most of these studies, which would have required an undetectable amount of TPNH.

The intracellular distribution of the lanosterol-reducing enzyme is similar to that found for the desmosterol-reducing system (6). The two systems are similar also in other respects. Both are TPNH-dependent and sensitive to —SH inhibitors, and they are similarly affected by the same inhibitors (triparanol, U-18,666A, U-5755). It is possible, therefore, that both reactions are catalyzed by the same enzyme—a "side chain reductase"—the activity of which is not strongly influenced by the configuration of the sterol nucleus. Results of the experiment showing the inhibition of reduction of C^{14}-lanosterol by added desmosterol are consistent with the above thesis, although they do not prove it.

The extent of conversion of lanosterol to 24,25-dihydrolanosterol under physiological conditions cannot be determined on the basis of the present study. It is clear, however, that rat liver can enzymatically reduce lanosterol and can also directly convert 24,25-dihydrolanosterol to cholesterol. The pathway of cholesterol biosynthesis in which side chain reduction is the very first reaction beyond lanosterol is therefore possible. On the other hand, Clayton and Bloch have shown that the C_{30} sterols formed from acetate in liver homogenates contain very little 24,25-dihydrolanosterol (15). Moreover, previous studies have demonstrated the potentiality for a pathway from lanosterol in which desmosterol is an intermediate and the final step consists of the side chain reduction (1, 16). However, most of the above studies demonstrate either a biological conversion of a postulated intermediate or an accumulation (or absence) of a postulated intermediate *in vitro* or under abnormal metabolic conditions and, therefore, do not establish its quantitative importance in the normal biosynthetic sequence. Experiments providing further information about this problem based on studies *in vivo* in normal animals are being reported separately (9).

SUMMARY

The anaerobic reduction of labeled lanosterol, biosynthetically prepared from 2-C^{14}-mevalonic acid, to 24,25 dihydrolanosterol has been demonstrated with rat liver homogenates. Enzymatic activity was associated with cell particles, mostly with microsomes, and required reduced triphosphopyridine nucleotide. The enzyme was completely inhibited on addition of *N*-ethylmaleimide or *p*-chloromercuribenzoate, and did not require a divalent cation for activity. Attempts to demonstrate the reversibility of side chain reduction of lanosterol during both anaerobic and aerobic incubations were not successful. Triparanol and two other inhibitors of cholesterol biosynthesis blocked the reduction of both lanosterol and desmosterol *in vitro*. Unlabeled lanosterol or desmosterol added to the incubation medium caused a comparable inhibition of reduction of C^{14}-lanosterol. It is possible that a single enzyme is responsible for the reduction of both sterol substrates.

Acknowledgments—The authors wish to thank Miss Ann E. Butler and Miss Margaret Dedeian for their skilled technical help in carrying out this study.

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